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ERRATA

In reference to an article, "Cardiotonic Glycosides: Some Aspects of the Chemistry and Pharmacology With Special Reference to Digitalis and Its Derivatives" by H. W. Paley, M.D., which appeared in TEXAS REPORTS ON BIOLOGY AND MEDICINE, Volume XIII, Number 4, pages 892–900, Winter, 1955, the guide lines and arrows from the titles of mixed glycocides at the sides at the left of the figures to the chemical configurations are in some ways incorrect. Anyone interested in obtaining a correct series of figures may contact H. W. Paley, M.D., University of Texas Medical Branch, Galveston, Texas.

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SALMONELLOSIS COMPLICATION IN HUMAN BARTONELLOSIS*

MANUEL CUADRA C.†

It is known that acute bartonellosis (Fiebre Grave de Carrion or Oroya fever) is frequently complicated by a severe febrile condition of septicemic type caused by salmonellas. The high mortality rate of the acute bartonellosis is due to this secondary infection. Before discovery of chloramphenicol, almost every case of bartonellosis complicated by salmonellosis was fatal. This secondary infection in most cases takes place during the convalescence stage, that is, in the period of recovery from anemia. In some cases it occurs "inside" the hematic phase of Weiss during the development of the febrile anemia.

Oroya fever has been known since 1870, when a severe epidemic developed among the workmen building the railway between Lima and Oroya. In 1885, Daniel Carrion, a medical student, inoculated himself with blood from a verruga nodule. In 21 days, he developed the disease, and died after 18 days from severe anemia.

In 1898, Barton (7) isolated a motile bacillus from the spleen of 5 patients whose death had been caused by "Fiebre Grave de Carrion." He thought that he had discovered the specific agent of the infection, but further experiences demonstrated it was not so. Many years later (8) he discovered the real agent which was named *Bartonella bacilliformis* by Strong (28).

Between 1904 and 1907, Biffi, Carbajal, Tamayo and Gastiaburu (11, 12, 32) stated that the motile bacilli were agents of secondary infection in the development of Oroya fever. In their opinion, these bacilli were the most important factor in the high mortality rate of Oroya fever. Such bacilli were also named "bacilos semiltíficos" because of its resemblance to the specific agent of typhoid fever.

In 1912, Barton (9) stated that the Barton's bacillus or "bacilo similtífico" had great influence on the progress of acute bartonel-

* Summary of "La complicación Salmonelósica en la Bartonelosis Aguda." Revista-Médica Peruana 307, July 1954. Accepted for publication by Texas Reports, Feb. 1956.

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losis not only because it modified the clinical picture and the course of the disease, but also because it was the causal agent of death in most patients with "Fiebre Grave de Carrion" (Oroya fever).

In 1932, Ribeyro (25) reported 19 cases of acute bartonellosis. Of these 6 died from complications with *Bacillus paratyphi B.* The author pointed that every case of Oroya fever complicated by this bacillus was always fatal, contrary to uncomplicated bartonellosis which had good prognosis. In 1935, Hercelles (15) isolated from the blood of 27 patients with bartonellosis: *B. coli* from 15, *B. paratyphi B* from 8, *B. paratyphi A* from 2, Eberth's bacillus from 1 and enterococci from 1. In 1938, Jiminez Franco (18) studied 25 autopsies of acute bartonellosis with the following results: tuberculosis in 8 cases, dysentery in 3, pneumonia in 2, typhoid fever in 2, cisticercosis in 1. Only 1 case was considered to be free of complications. In 1940, Alzamora Castro (3, 4) reported 12 fatal cases of salmonellosis from a group of 31 bartonellosis patients. According to this author death from bartonellosis alone is rare. In 1948 L. A. Aldana G. (2) reported 6 fatal cases of acute bartonellosis complicated by *Salmonella enteritidis.* He concluded that this was the most frequent species of complicating salmonella.

Ricketts (26) reported that intercurrent infections were found in 47.6 per cent (50 cases) in a group of 105 patients showing 3 stages of human bartonellosis: "Hemolytic febrile anemia stage," "preeruptive stage" and "eruptive stage." These infections were: *Salmonella typhosa* 5, *Salmonella schottmülleri* 2, anaerobic intestinal septicemia (organisms undetermined) 3, amebic colitis 3, other intestinal parasites 12, malaria 28, tuberculosis 3, syphilis 1, pneumococci 1, puerperal infection 1 and *Brucella melitensis* 1. He says "It appears from these data that in cases of *Bartonella baciliformis* anemia, the enteric infections are fatal in almost every instance, as but 1 out of 12 survived." The mortality in 105 cases was 23 (22 per cent). According to Ricketts, blood cultures are very helpful in establishing the intercurrent salmonella infections while agglutinations are very unreliable. He did not mention spleen culture of cadavers which is as important as blood culture.

In 1954, Colichón (14) for the first time applied serological

and biochemical methods in accordance with Kauffmann-White table. The results obtained in 13 patients indicated salmonellas of animal source, *Salmonella typhimurium*, the most frequent species found.

Stage of Bartonellosis in Which Salmonella Complication Takes Place. A typical case of human bartonellosis presents two successive manifestations or syndromes, the hemolytic febrile anemia stage and verrucous stage, the former called by many authors, Oroya fever. Odriozola (23) called it "Fiebre Grave de Carrion." It is characterized by *Bartonella bacilliformis* invasion of red blood cells, therefore Weiss called it "hematic phase." The period of convalescence from this stage begins with fall of fever, gradual disappearance of *Bartonella bacilliformis* from red blood cells. Human bartonellosis anemia is of short duration with two successive periods, one of red cell destruction followed by regeneration. Chronic type of human bartonellosis anemia has not been observed. These two stages of anemia also occur in murine bartonellosis anemia, however murine anemia with hemoglobinuria is more severe than human anemia where hemoglobinuria is not observed. Relapse of anemia in human bartonellosis, such as frequently occurs in murine bartonellosis, has not been observed, however the possibility of its occurrence in exceptional cases is not to be overlooked.

From the clinical standpoint, human bartonellosis may be seen in three forms: 1) Febrile acute anemia (Oroya fever or Fiebre Grave de Carrion), not followed by verrucous eruption. 2) Febrile acute anemia followed by verrucous eruption. 3) Verrucous eruption not preceded by febrile acute anemia. Of these, the first and third forms are most frequently observed. The second form at present is less prevalent than in the past. In many instances, patients have been retained in the hospital for 1 to 5 months in order to watch for the appearance of the verruga nodules, however eruption did not appear, perhaps due to antibiotic therapy.

From the standpoint of salmonella complication, we consider only the febrile anemia stage (Oroya fever or hematic phase of Weiss). The intercurrent salmonella infections (Table 1) may take place (a) "Inside" this stage (Fig. I). In this case, both organisms, bartonella and salmonella, coexist (precocious type or

TABLE 1

The Course of Oroya Fever with Salmonellosis Showing Its Relation to Red Cell Count and Percentage of Cells Parasitized with Bartonellas

Case	Termination of Oroya fever (day)	Beginning of Salmonellosis	Red Blood Cells, Per Cent of r.b.c. Parasitized In Oroya fever stage	At Initiation of Salmonellosis	Stage of Oroya fever in which Salmonellosis Takes Place	Treatment: Results
1	15th	7 days later	1,500,000 80%	2,980,000 0%	Convalescence	Chloromycetin Cured
2	9th	7 days later	3,370,000 40%	3,260,000 1%	Convalescence	Chloromycetin Cured
3	11th	6 days later	1,000,000 100%	2,080,000 5%	Convalescence	Penicillin, Streptomycin Died
4	10th	40 days later	1,050,000 15%	4,000,000 0%	Convalescence	Chloromycetin Cured
5	17th	1 day before	1,020,000 90%	1,830,000 30%	End of acute phase	Chloromycetin Cured
6	16th	8 days later	1,640,000 90%	2,890,000 4%	Convalescence	Chloromycetin Cured
7	No end	"Inside" Oroya fever	1,580,000 100%	? ?	"Inside" Oroya fever	Furadantin, Aureomycin Died
8	No end	"Inside" Oroya fever	1,180,000 98%	? ?	"Inside" Oroya fever	Achromycin Died
9	13th	5 days later	1,510,000 90%	2,380,000 4%	Convalescence	Chloromycetin Cured
10	16th	9 days later	3,400,000 50%	2,580,000 2%	Convalescence	Chloromycetin Cured
11	No end	"Inside" Oroya fever	1,550,000 95%	? ?	"Inside" Oroya fever	Ilotycin Died
12	18th	4 days later	1,100,000 95%	2,800,000 0%	Convalescence	Streptomycin Died
13	18th	6 days later	1,800,000 100%	3,470,000 0%	Convalescence	Chloromycetin Cured

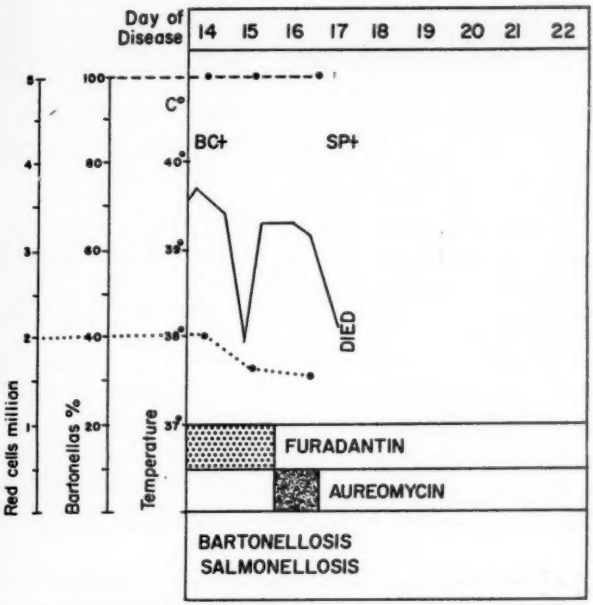

FIG. I, Case 7. Salmonella complication occurs "inside" the Oroya fever; both organisms *Bartonella bacilliformis* and salmonella coexist; severe anemia without signs of hematic regeneration; *B. bacilliformis* abundant in peripheral blood; blood culture for salmonella positive.

early type). Large quantities of bartonella organisms are found in the peripheral blood; blood cultures for salmonella are positive; there is severe anemia of macrocytic type associated with a relatively low per cent of reticulocytes (no hematic regeneration); the prognosis is poor. (b) Secondary infection may take place during the first week of convalescene ("intermediate type"). In this case, there are no bartonellas in red blood cells or they may be present in small coccoid forms. Blood cultures for salmonella are positive; the anemia is severe but signs of hematic regeneration are present, such as, high per cent of reticulocytes, macrocytosis, and anisocytosis; the prognosis is poor (Figs. II and III). (c) Intercurrent salmonella infection may appear long after

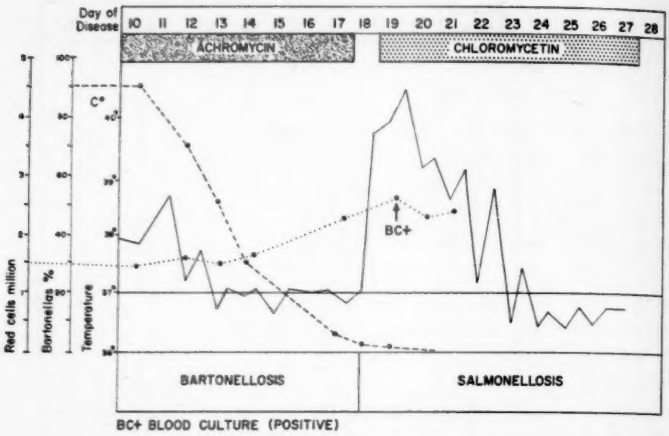

FIG. II. Case 9. Salmonella complication appearing in the convalescence stage of Oroya fever; *B. bacilliformis* is rare in the peripheral blood; severe anemia with marked hematic regeneration; blood culture for salmonella organisms positive; typical response to chloramphenical with recovery of patient.

Oroya fever is over (a "late type") when the patient is almost well. In this case there are no bartonella organisms in peripheral blood; the anemia is slight (residual anemia); blood cultures for salmonella are positive; the prognosis is relatively favorable. In one case (Table 1, case 4), secondary infection took place 40 days after the anemia stage.

It is not known whether or not secondary infection may take place in cases of verrucous phase not preceded by the Oroya fever.

Symptomatology

The 13 patients studied for salmonella complication offered a typical clinical picture of Oroya fever. These were all natives of Peru with an age range between 15 to 42 years, the majority over 20. All the patients came from endemic zones of bartonellosis and entered the hospital with acute bartonellosis symptoms. The red cell count in 3 patients was 3 million and in the remaining 10 patients, about 1.5 million. The red blood cells were 80 per cent parasitized in 10 of the cases and from 15 to 50 per cent in the remaining 3. The duration of sickness before entering the

hospital was 6 to 15 days. The outstanding clinical manifestations were: a) Fever. b) Disturbances of the digestive system. c) Prostration.

Fever. The onset of the salmonella complication taking place during convalescence from Oroya fever is marked by a temperature rise following a chill in the majority of cases. It is moderate on the first day becoming high on the second day, a more rapid rise than typhoid fever. Temperature remains high during subsequent days with morning remissions, and may present different types, continuous, remittant or irregular. Aldana (2) pointed out that before chloramphenicol, the continuous type was most frequently found, however in our cases, the remittant type was more prevalent.

Uncomplicated bartonellosis is accompanied by fever of moderate degree, rapid anemia being the most prominent feature, therefore, a high fever in the course of the disease must be suspected as due to salmonellosis.

Chills are very frequent sometimes as intense as in malaria,

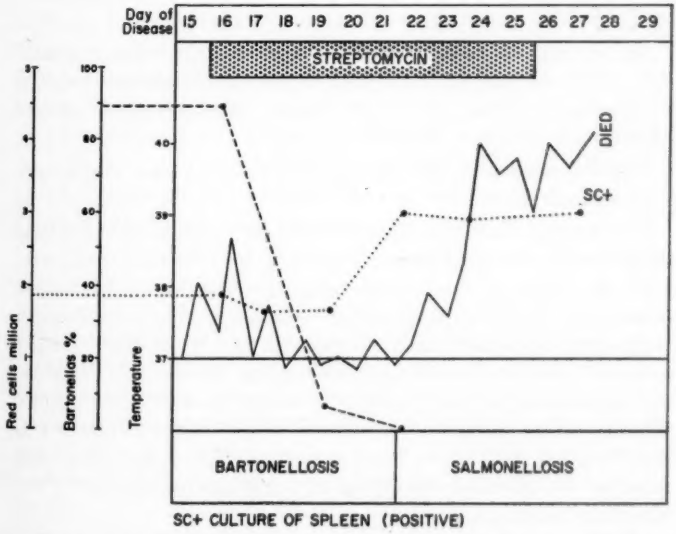

FIG. III, Case 12. Salmonella complication in the convalescence stage of Oroya fever; patient not treated with chloramphenicol resulting in death.

appearing at the onset of the secondary infection and during its course. Nocturnal sweating is also frequently observed, in some instances as profuse as in malaria or brucellosis. Fever disappeared generally by lysis in 8 surviving cases treated with chloramphenicol(chloromycetin Parke, Davis & Co.), whereas 5 fatal cases not treated with chloramphenicol (Table 1) showed high fever shortly before death.

It is difficult to determine the duration of fever in cases spontaneously recovering, as these cases are rare. Reviewing medical literature, however, it was possible to find data on the duration of fever in fatal cases without specific therapy, as shown by 15 cases diagnosed by blood culture: Julian Arce (5), Tamayo (30), L. Aldana (1), L. A. Aldana C. (2), Cuadra and Colichón (14). According to these authors, the intercurrent Salmonella infection produces death on an average of 7 days. In 1948, we observed a patient with a blood culture of *S. enteritidis*, who in convalescence from Oroya fever suddenly developed high fever, and died vomiting blood 48 hours later. In one case of the patients of Aldana (2), death occurred 12 days after the onset of secondary infection. We consider these 2 cases exceptional.

In cases in which intercurrent infection takes place "inside" the course of Oroya fever (bartonellosis and salmonellosis), it is impossible to know when this begins. Our fatal cases 7, 8, and 11 belong to this type (Table 1).

Other symptoms besides fever are: headache, pain of eye balls, muscles and joints.

Disturbances of the digestive system. Generally digestive disturbances are not intense, appearing in most cases some days after the onset of fever, consisting of abdominal pains, either diffuse or localized. Abdominal inflation was often observed, frequently associated with severe pain and discomfort. Nausea and vomiting are common manifestations. The number of stools are in most cases, 1 or 2 every 24 hours, fluid or pasty in consistency. Only in case 4 (S. typhimurium) did we observe true diarrhea. None of our patients presented profuse diarrhea, cholera-like, as commonly observed in acute gastro-enteritis by salmonella food poisoning.

The tongue was normal, only case 13 presenting a typhoid-like appearance. Mild jaundice of short duration was observed in

6 of the patients, appearing several days after the onset of the intercurrent infection and accompanied by a slight enlargement and tenderness of the liver. Van der Bergh reaction indicated predominance of direct fraction of bilirubin, completely different from that of hemolytic nature which appears as a result of bartonellosis only.

When hematic regeneration was in progress as in cases 1, 2 and 9 (Table 1), where intercurrent salmonella infection took place after *Bartonella bacilliformis* had disappeared from red blood cells, we may conclude that jaundice was due to liver damage by salmonella. Jaundice as a result of both bartonellosis (hemolytic jaundice) and salmonella (liver damage) is shown in cases 7, 8 and 11 where secondary infection took place "inside" Oroya fever with 80 per cent of the red blood cells parasitized, resulting in hemolytic anemia. It is difficult to give a correct interpretation of these 3 cases because of the low quantity of bilirubin although a tendency to parenchymal type of jaundice may be suspected.

We deduce that jaundice with direct bilirubin predominating indicates salmonella infection while jaundice with predominance of indirect bilirubin is caused by bartonella. However the possibility of associated salmonellosis should not be excluded.

Prostration and general appearance of salmonella complication. Secondary infection by salmonella in the course of acute bartonellosis is a severe infectious disease. Symptomatology begins with malaise and fever of moderate degree, becoming rapidly malignant, with death occurring usually on the seventh day, sometimes on the second, but seldom later than the tenth day after the onset. Pallid face, as a result of bartonellosis anemia, high temperature, marked sweating, accelerated respiration, frequent and weak pulse, accentuated prostration and expression of great distress indicate grave toxemia. Except in rare instances, there is no apathy and stupor as observed in typhoid fever. The patient feels pain over the whole body, especially in the abdomen. Nausea and vomiting are frequent. Delirium is often noted and later, death may be preceded by semicomatose or comatose state.

The clinical appearance of salmonella complication differs from classical typhoid fever by almost sudden onset of fever,

rarity of stuporous state, rarity of the enlargement of spleen, frequent chills, tongue rarely dry, pulse rate very accelerated, nocturnal sweating, and frequently a remittent type of fever. In addition, salmonella may be easily isolated from patient's blood. In spite of this, salmonella complication has the character of general infection such as typhoid fever and resembles this in some symptoms, *e.i.* the abdominal distention and the tendency to diarrhea.

On the other hand, salmonella complication because of its violent character with sudden onset, its tendency to nausea, vomiting and abdominal pain, resembles the manifestations of salmonella food poisoning in which there is only local attack of the digestive tract without invasion of the blood. We suggest that salmonella infection resulting as a complication of bartonellosis from the standpoint of symptomatology and pathogenesis, might be considered as intermediate between food poisoning (local infection) and typhoid syndrome (general infection).

Blood

Salmonella complication takes place in patients with anemia. If it occurs during the development of Oroya fever stage, the secondary infection coincides with the red blood destruction stage. If the intercurrent infection takes place in the convalescence stage of Oroya fever, it coincides with the regenerative stage of red blood cells (low percentage of bartonella or total absence of them and high reticulosis). This second complication seems the most frequent (Table 1).

Hurtado, Pons and Merino (17) stated that salmonella complication reduces the number of reticulocytes, which may disappear shortly before the patient's death. Our observations agree with these authors in the sense that secondary infection produces reduction of the number of reticulocytes; therefore, and as a consequence, the red blood cell level and hemoglobin remain stationary or decreased. After stopping intercurrent infection by chloramphenicol treatment, the percentage of these cells rises.

From our 13 patients, in 2 cases, 2 and 10, the anemia was not severe. The lowest red cell level during acute bartonellosis was above 3,000,000 erythrocytes per ml. however when the sec-

ondary infection occurred it assumed similar severity to those cases which had suffered intense anemia (Table 1).

According to Hurtado, Pons and Merino (17) the acute bartonellosis presents in most cases a normal leukocyte count and, in some cases, leukopenia. We have observed the same in our patients prior to appearance of intercurrent salmonellosis. At this time, the number of leukocytes remains unaltered with a few cases showing leukocytosis or leukopenia. Some physicians believe that high leukocytosis indicates presence of complication.

In regard to hemogram, the leukocytes follow the three stages described by Shilling for infectious diseases. If the intercurrent salmonellosis occurs in the Oroya fever stage, the usual lymphocytosis is replaced by neutrophilia. If the patient survives secondary infection, the lymphocytosis reappears (50–60 per cent).

Salmonella complication: occurrence and mortality rate. As to the frequency of salmonellosis, we found 14 cases among 38 studied: 38.25 per cent.

Mortality rate in Oroya fever has always been considered high, because it has been accepted that this high mortality was due to the bartonellosis. According to our experience, the most important cause of the high mortality rate is secondary invasion by salmonella. Contrary to other reports that many kinds of secondary infections may occur in the course of acute bartonellosis, in our opinion, the only important secondary infection is of salmonella etiology. Other infections are rare and of no practical importance. We emphasize that diagnosis of death caused by Carrion's disease should be based on bacteriological examination. Because there are no descriptions of specific histological lesions in this infection, it is impossible to find the cause of death based on clinical appearance and on macroscopical and microscopical examinations of organs only. Many investigators have described tissue lesions as a consequence of bartonellosis (Strong (29), Weiss (37), Mackehenie (19) etc. with no previous exclusion of secondary infection. We feel that the pathology of Oroya fever must be revised.

From 57 cases of salmonella complication reported by different authors, 52 died, a mortality rate of 91.22 per cent (before chloramphenicol).

Pathogenesis of Salmonella complication. It is difficult to

establish the reason for the frequency of salmonella complication in acute bartonellosis patients, therefore only the factors which make possible the development of secondary infection will be enumerated.

Ribeyro in 1932 (25) applied the ideas of Charles Nicolle (21) on the origin and phylogenic relationship of bacteria to the study of bartonellosis. According to Nicolle the exanthematic typhus has three forms: first, the Proteus X 19 which represents the saprophytic form; second, the *Rickettsia prowazeki* form of moderate pathogenicity; third, a virus of maximum pathogenicity. Ribeyro believed that the salmonella was to *Bartonella bacilliformis* as Proteus X 19 was to *Rickettsia prowazeki* with the difference that *S. paratyphi B* is pathogenic, contrary to the non pathogenic proteus. He also speculates on *S. paratyphosa B* as the ancestral origin of the bartonella. However contrary to the positive Weil-Felix test of typhus, Ribeyro found that serum from bartonellosis patients did not agglutinate *S. paratyphosa B.* Today we know that *S. typhimurium* is the most frequent invader. *B* was the usual species invader, stated that *Bartonella bacilliformis* and *S. paratyphi B* had common antigen. Aldana (2) re-

In 1948, Tisnado (33) believing as Ribeyro that *S. paratyphi* ported 6 fatal cases of salmonellosis complication, stating that the secondary infection was produced at the end of the anemic febrile phase or in the convalescence period. He theorized that the RES blockade during the anemia and destruction period of red cells may lower the defense forces enhacing salmonella invasion.

Although Weiss (37, 38, 39) has not made special studies on salmonella complication of bartonellosis, he has sometimes referred to the infection complications in general, which are "frequent in this disease." The author maintains that human bartonellosis develops in two successive stages: hematic stage. and histoid stage. In the first, bartonella attacks blood (septicemic stage) producing typical cases, acute anemia with bartonella in the red blood cells. After a short time, immunity is produced causing lowering of fever, gradual disappearance of bartonella from red blood cells. This last phenomenon marks the beginning of the second or histoid stage when bartonella attacks fixed tissues especially RES and the disease culminates with verrucous

eruption. From the immunologic point of view, according to Weiss, the immunity forces fade at the end of the hematic stage or the beginning of the histoid stage. This is the most critical moment for the patient. In the great majority of cases, death occurs at this stage because of the bartonellosis itself, or from the secondary infections starting at this phase.

From our experience there seem to be a number of factors which make salmonella complication possible. Patients who come to hospitals in Lima with acute bartonellosis acquired in endemic zones were not born in those regions. Usually coming from the Sierra of Peru where this disease does not exist, they visit the endemic zones where they acquire bartonellosis.

The little Sierra villages are mainly composed of small scale farmers and cattle breeders. Drinking water comes directly from springs or small rivers where the regional cattle also drink. Excreta are left in open air, near homes, in the streets or in the fields. Domestic animals walk freely in the streets and their fecal material is found everywhere on the sidewalks, in the patios, etc. In the homes, dogs, cats, chickens, ducks and pigeons live together, and in the kitchen, artificial burrows are found full of guinea pigs. House flies complete the picture. No attention is paid to babies crawling on the floor eating earth.

In such conditions, with human life on an animal level, no one can escape salmonella infection. Small babies because of their great susceptibility as demonstrated by Hormaeche (16) are the frequent victims in which the different syndromes are found. Weak children die in the battle but the strong survive and gain immunity against future aggressions, but become at least temporary carriers of salmonella. When these people acquire bartonellosis, they lose their immunity against salmonella and assume again the susceptibility of the child.

Even though many Peruvian physicians accept the theory that acute bartonellosis patients are sensitive to many secondary infections, we believe that they are sensitive only to salmonella. These physicians base their opinions on clinical diagnosis, not on bacteriological data. Salmonella can produce many different clinical syndromes, a phenomenon which gives the impression of etiological multiplicity. Such syndromes can be: typhoid-like, septicemic, acute gastro-enteritis, light diarrhea, dysentery-like,

meningitis, broncho-pneumonia, pleuritis, pericarditis, otitis, pharyngitis, endocarditis, etc (16).

It is significant that in exanthematic typhus, which is a frequent disease in the Sierra of Peru, salmonellosis as complication of the primary disease has not been described. Proteus, on the other hand, has been isolated from blood, organs and urine of typhus patients (27, 34). It seems therefore that salmonella has a selective preference for bartonellosis.

It is unknown why bartonellosis always destroys the defense which the adult has against salmonella invasion. Nevertheless the phenomenon is similar to the occurrence of proteus in exanthematic typhus patients with the difference that proteus is not pathogenic as compared with the pathogenicity of salmonella. Investigations in the field of murine bartonellosis give interesting data in this regard: Pittaluga (24) points out that pasteurella and salmonella invade the blood of rats in which, by splenectomy, bartonellosis has been produced. Battistini and Weiss (10) report on frequent cultivation salmonella in their attempts to cultivate bartonella.

Treatment. Eight of our patients were treated with chloramphenicol (Chloromycetin Parke-Davis) and all survived; the remaining 5 were not treated with this antibiotic and died (Table 1).

Aldana (2) in 1948, before the discovery of chloramphenicol, reported 6 cases of complication with *Salmonella enteritidis.* Five of them were treated with penicillin and one with methylene blue without success.

In regard to bartonellosis alone, we do not have scientific evidence that chloramphenicol or any other antibiotic (penicillin, streptomycin, aureomycin, ilotycin, achromycin, terramycin, etc.) is effective because no trials have been made except by Payne and Urteaga (35) who suggested that chloramphenicol may be effective.

In our opinion, it is not possible to establish with scientific certainty that antibiotics are effective in bartonellosis. Such assays are usually made in Lima where the patients with bartonellosis arrive in most cases during the late stage of Oroya fever, very few cases appearing during the initial phases of the disease. As a consequence it is impossible to know whether the recovery was

spontaneous, by immunity, or because of antibiotics. We believe that trials should be done in patients within the first 5 or at the maximum 7 days of acute bartonellosis with fever, bartonella in bacillary form, anemia in initial phase, and normal reticulocytes. Only then will it be possible to know whether or not the antibiotics change the course of the disease. Meanwhile we can affirm that the effectiveness of antibiotics in Oroya fever may be considered as a possibility, but not as certainty. According to Franco (18), Castro (3) and contrary to high fatality rate in salmonella complication, uncomplicated bartonellosis has a low mortality rate. We agree with this opinion.

At present chloramphenicol is the most suitable antibiotic available for the treatment of acute bartonellosis because it is the only effective drug against the intercurrent salmonella infection.

It is important that physicians recognize that many patients are admitted to hospitals with double infection, bartonella and salmonella. If, under these circumstances, the patients are not treated with chloramphenicol, death may occur (patients 7, 8, 11, Table 1). Patient 7 with *S. dublin* died on the 16th day after onset with bartonellosis with 90 per cent red blood cells parasitized with *Bartonella bacilliformis.* He was treated with furadantin and later with aureomycin without success. It was thought that the patient had died from bartonellosis and that aureomycin had been ineffective, but this proved untrue when Colichon identified *S. dublin* (14) isolated from the organs at autopsy. Patient 8 died 14 days after onset of bartonellosis; 80 per cent of the red blood cells were parasitized with *Bartonella bacilliformis;* he was unsuccessfully treated with achromycin and later penicillin; blood culture was positive for *Salmonella typhimurium* which was also isolated from organs at autopsy. Patient 11 died 16 days after the onset of bartonellosis; 90 per cent of the red blood cells were parasitized with *Bartonella bacilliformis;* treatment by intravenous injection of ilotycin was ineffective; *Salmonella typhimurium* was isolated from organs at autopsy.

Bartonella bacilliformis may lead to acute anemia which can be controlled by blood transfusions until the antibody level can produce a natural defense against the disease. In case of salmonellosis, if chloramphenicol is not given, death is almost certain

to occur. Consequently, if chloramphenicol is used during the Oroya fever stage, then the feared salmonella complication will not appear during the convalescence stage. This may be considered as a preventive measure.

Summary. Oroya fever (acute bartonellosis) is frequently complicated by a severe febrile condition of septicemic type caused by salmonella organisms. It occurs in approximately 40 per cent of patients. While uncomplicated Oroya fever has good prognosis, cases complicated by salmonella are 90 per cent fatal. Most salmonella isolated from patients are of animal source with *Salmonella typhimurium* the most frequent species. Salmonella complication takes place generally in the remission stage of Oroya fever, but it may occur early "inside" this primary condition. The pathogenesis of salmonella complication is unknown. Some theories about it have been discussed. Chloramphenicol is greatly effective against the grave danger of salmonella complication.

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THE PERSISTENCE (SURVIVAL) OF MICROORGANISMS: III. IN CULTURE MEDIA

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One of the foremost problems in the field of microbiology has been the culturing and storage of organisms in the laboratory in such manner as to maintain viability as well as their characteristics of morphology, metabolism and virulence. A multitude of reports on this subject have been made from myriads of experiments with more or less unanimity of results. The factors involved in the storage of cultures are numerous. Several main methods have been used for maintenance of stock cultures: (1) low temperature storage; (2) drying by lyophilization or other procedure; (3) exclusion of air and maintenance of moisture, and (4) use of a combination of the various methods.

The factors involved in survival may be discussed in general as to environmental factors or organism characteristics.

Environmental Factors Affecting. The term "culture media" is used loosely here to cover the survival of organisms *in vitro* in media of all types whether liquid, solid or dried, in various containers under experimental conditions primarily in the laboratory.

The medium in which the organism is grown or stored plays an important role in determining the length of survival and maintenance of characteristics of organisms. The presence of inorganic buffers protects against extreme pH changes but may on occasion be toxic for some organisms. If fermentable carbohydrates are present, toxic acid, aldehyde, alcoholic or other end products might slowly pile-up to kill the organism. Some salts are necessary for osmotic effect but can be toxic if in large concentrations in the medium. The organic substances in the medium may supply buffering capacity against pH and other

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changes but may provide in metabolism a source of toxic end-products.

The physical state of the medium may affect survival time of organisms. Survival in liquid, on solid surface or in dried state may vary with certain organisms. The amount of medium may offer protection against physical forces of temperature and radiation or oxidation-reduction potential changes which might be detrimental to some of the anaerobes particularly.

The temperature of storage is one of the most important factors in determining length of survival. Organisms maintained at temperatures which allow metabolism of the organism to take place will not only produce toxic, limiting end-products but will age to become more susceptible to detrimental action of physical and chemical forces. Usually the higher the temperature the more rapid the death rate. Low temperature of storage obtained in refrigerators (approximately 5° C.) prevents active metabolism of most microorganisms and serves well in maintaining numbers and general characteristics of organisms. Even lower temperatures have been used, ranging from —5° C. (deep-freeze) to —75° C. (dry ice) to temperatures of liquid oxygen. In studies below the freezing point, the rate of freezing is a factor in the survival of organisms. Usually rapid freezing allows greater survival. Repeated freezing and thawing destroys many organisms probably through rupture of cell walls by formation and dissolution of the ice crystals. Presence of protein in concentration protects against such destruction.

Desiccation is usually considered as destructive to most organisms. Maintenance of cultures at low temperatures prevents loss of water content through evaporation. Materials such as cultures at room temperatures (25° C.) or incubator temperature (37° C.) may be sealed or covered by various means including wax or rubber stoppers or screw-caps. Lyophilization, drying from the frozen state, has been exploited as a means of maintaining organisms. The suspending medium, rate of freezing, rate of drying and subsequent storage method are important factors in the success of the procedure. Usually a protein-suspending medium with rapid freezing and rapid drying with the material then being sealed off under vacuum and stored at refrigerator or colder temperature, gives the best results. The total dryness

affects survival since a small amount of water in the end-products allows deterioration. In some instances, inert gas such as nitrogen has been used instead of keeping organisms under vacuum. Storage may or may not be at low temperature. The lyophilization procedure may result in the destruction of many organisms in the preparation, but those remaining viable retain their characteristics for long periods without throwing off variants.

Radiation of various types affects survival of organisms in cultures. Storage of cultures in the dark away from direct or diffuse daylight allows longer survival. Exposure of cultures to artificial sources of UV or of other radiation not only increases the death rate but increases the development of aberrant forms as mutants.

Removal of oxygen and substitution of an inert gas will prevent metabolism and allow long storage. The use of sterile mineral oil over cultures prevents desiccation and excludes oxygen as well, thus slowing down metabolism and allowing survival for long periods.

Many studies have been made on these various factors affecting survival for better maintenance of microbial cultures. The cultures have also been exposed to extremes of temperature, desiccation, pH, eH, radiation, chemicals and pressure to determine the ability of different organisms to withstand these forces. Some of these studies are reported here.

Organism Factors Affecting. The survival of an organism in culture media is not only dependent upon the media and other environmental factors, but also upon the intrinsic characteristics of the particular organism itself. The genus, species and strain are important in that they may have characteristics providing resistance to physical and chemical forces. The presence of spores, capsules, especially rigid cell walls or other cellular components may aid in survival. The presence or development of more resistant variants or mutants may play a role in persistence. The rate and type of metabolism plays a part also in the pile-up of toxic end-products which might destroy the organism more rapidly. In all survival studies the number of organisms exposed and the age in the growth curve at time of storage or exposure are important factors in the survival or persistence of organisms.

Summary

•*Bacillus anthracis* (Table C1). Dried cultures survived 4½–35 years with good immunizing property; low temperature kept virulent forms alive for 8 years. In liquid form survival up to 1877 days in glycerin-serum at room temperatures. In sunlight killed in hours. May live for 11–14 years at 5–10° C. Liquid air killed in 6 hours, liquid hydrogen 10 hours. On solid media viability apparently low. May survive sun for 1½–4½ hours depending on season.

•*Bacillus species* (Table C2). Dried cultures survive for 4–5 years. Liquid cultures for long periods. Spores resist sun for 5–6 hours. *B. globigii* was found to be resistant to heat, gentian violet and streptomycin. On solid media 8 month survival. At freezing temperature over 80 week survival noted.

•*Bacteriophage* (Table C3). Dried typhoid phage lived for 26 years, others for 3 years. Resistance to heat aided by dryness. Freezing and thawing destroys phage. Lyophilization of dysentery phage inactivates much of the activity. Sunlight is toxic. Coliphage may be active for 7–17 years. When dysentery phage dried, no loss in 6 months. Phage resistant to pH changes.

•*Spirochetes* (Table C4)

Borrelia—At —78°C. the organisms survive for a year. Lyophilizing destroys many cells and may live only 192 hours.

Leptospira—Ten month viability in tissue broth at —78° C. In temperatures of 29–42° C. survival varies upwards from 5 days to 16 months.

Spirillum—In mouse blood at —78° C. for 1 year.

•*Brucella* (Table C5). When dried, cultures live 4–5 years. In liquid viability ranges from 15 days to 400 days. On solid media no loss in 8 days. On storage colony forms change. Storage at 37° C. shows 4 year survival. Sunlight and drying lowers disease incidence. In lyophilizing high temperature lowers recovery while slow rate of drying gives better survival. Storage at 2–5° C. yields 80 per cent viable in 100 days.

•*Clostridium* (Table C6). Dried cultures live for 3–5 years. Organisms survive lyophilizing. In liquid form exposed to various changes of temperature, pressure and pH fair survival is listed. On solid media *Cl. tetani* lived for 38 years. *Cl. botulinum* showed 140 day survival at 5° C. The spore stage of these organisms protected against high temperature and sunlight for short periods.

•*Corynebacterium* (Table C7). In the dried state 4–5 year viability found. The organisms resist sunlight when dried. In liquid media 6 month survival is usual. Organisms resist drying and sunlight well. On solid media 7–18 month viability reported.

•*Erysipelothrix* (Table C7). When dry the cultures live for 4–5 years.

•*Diplococcus* (Table C8). In dry form the organism may exist for 4–8 years depending on temperature of storage. Lyophilized strains show good survival numbers for at least 3 years. Liquid cultures live over 6 months. On solid media viability of 50 days–3 months is listed. Low pH is detrimental as are temperatures above 56° C.

•*Escherichia coli* (Table C9). Survival in dry state of 4–5 to over 10 years is listed. Early studies showed shorter periods. Freezing and thawing for

lyophilizing destroyed many. In liquid exposed to increased temperature, freezing temperatures and chemicals, short periods of survival (in hours) are shown. In ordinary cultures at room temperature, viability over 1 year is found. Increasing salt concentrations destroy the cells. Very low temperatures destroy some cells rapidly (—195° C.). A temperature of 60° C. with high humidity is resisted for 7–10 hours. On solid media survival may to over 11 month survivals are listed. Sunlight during various seasons is resisted for 1½ to 4½ hours. Viability for 14 years noted in one instance at R.T. in dark. Wide variations in temperature affect survival adversely.

•*Microorganisms* (Table C10)

Alcaligenes—In dried form, survival of 4–5 years is reported.

Aerobacter—For 4–5 years in dried state, 31 days dried on paper at 37° C. Reports suggest the organism resists alkaline pH.

Hemophilus—Various species including *H. pertussis* lived for 5 years in dry state. In blood broth at —15 to 20° C. a few hours. On agar for 4–8 months.

Klebsiella—When dried, survival of 4–5 years listed, in serum at 37° C., 43 days, while in sealed tubes on agar 12–13 year viability has been demonstrated.

Lactobacillus—At 37° C. in dry state poor survival but at lower temperatures 3–4 year survival is shown. It resists liquid hydrogen for 7 hours and —10 to —80° C. for long periods. Rapid freezing aids survival. On solid media, 2-year viability was found.

Proteus—When dried, 4–5-year survival. In liquids good survival was reported. On solid media, 8 month viability found for some, 4–5 years for others and 19 years when on agar in sealed tubes at R.T.

Pseudomonas—Survival similar to Proteus, 4–5 years when dried, in liquid and on agar good survival found. It is slowly destroyed by pH up to 11.5.

Flavobacterium—In liquid media at —5 to 15° C., survival of less than 77 days revealed.

Achromobacter—Grows at low temperatures (0° C.).

Azotobacter—Viability of 10 years on dried dextrin agar is reported.

Malleomyces—At low temperatures of 1–4° C. under vacuum, 25 month survival is listed.

Erwinia—Low RH aids survival in exudates.

•*Microorganisms (General)* (Table C11). Generalizations on effects of temperature, freezing, drying are given. Better survival with covering of paraffin oil is indicated.

•*Mycobacterium* (Table C12). In dried state reports vary from 6–12 months to 4–5 years and one report of 17 years following vacuum desiccation. In liquid preparations, reports of several months to several years are reported. On the various isolation media, 4–8 month survival up to 6 years is listed. Low temperatures gave poor viability. Drying gives good survival. Low pH destroys but not too rapidly.

•*Neisseria* (Table 13). These organisms are very sensitive to drying and sunlight. Under freeze-drying conditions, 4–5 years of survival listed and

up to 18 years in others, but in nature in sunlight only a few hours may kill. Liquid cultures of the meningococcus at low temperature (frozen) survive for months and up to 2 years. In serum, 16 month survival is listed. On solid media it lives for 8–27 weeks at low temperatures. The gonococcus survival is poorer except when dried, 4–5 years up to 18 years. In liquid media 7–8 week survival is listed, pH of 7.4–7.6 allow best survival. On solid media, 8 month viability is given.

•*Pasteurella* (Table C14). Dried materials live 3 to 4 days when not in sun, 3–4 hours in sun. When dried in lab for stock, 4–5 year survival obtained. Liquid materials of *P. pestis* may survive a few months at freezing temperatures. Solid media survival of 20–25 years is reported. *P. tularensis* may live for months in frozen conditions. Other Pasteurella strains have somewhat similar persistence characteristics.

•*Protozoa and Metazoa*

Entamoeba—Three day to 3 week viability in Ringer's and other solutions and 10 days in powdered starch medium.

Plasmodium—In chicken red cell suspension, 72 hours without loss.

Trichomonas—Survival of 4–13 days at 27–37° C. in media; at low temperature, 2–3 week survival. Only 6 hours when dried.

Trypanosomes—In blood agar tubes, various species lived for 3–4 months at 25° C.; survival varied at freezing temperature of a few hours to a few months.

Schistasoma—Ten to 18 day persistence *in vitro* in serum.

Leishmania—Four month persistence in blood agar at room temperature.

Ascaris—Freezing temperatures inactivated in 6–20 days; high temperatures (60–70° C.) destroyed in a few minutes.

•*Rickettsia* (Table C16). For lyophilizing, sucrose was found effective. At room temperatures survival of a few hours to 1 week are recorded; at refrigerator temperatures, 2 week viability is listed while at freezing temperatures near —20° C., several month viability is found. With glycerol added, 10 month at —10° C. is reported.

•*Salmonella species* (Table C17). When dried at natural temperatures several hours of viability is found but lyophilizing allows viability of 4–5 years or as long as 10 years. In liquid cultures, 3–4 weeks is the usual report with occasional suggestion of 12 month viability. On solid media survival of nearly 2 years on blood gelatin and 98 day viability on gelatin are reported.

•*Salmonella typhosa* (Table C18). When desiccated, thin layers survived 5–15 days and thick layers lived for months. In liquid media such as saline survival over 6 days was found, at —20° C., 4½ months are listed and 10 years in tryptic digest. Resistance to liquid air, ultra violet, heat and freezing are given. On solid media, 91 day to over 8 month resistance was found. Other survival of 3 years to 8 years is listed on artificial media at room temperatures while lyophilized strains survived for about 4 years.

•*Serratia* (Table C19). Lyophilized strains lived for 4–5 years. In liquid, marscescens may survive for 20 years. Centrifuging destroys cells as well as radiation from the sun.

•*Shigella* (Table C20). Desiccated organisms may live only 20–25 days, but

lyophilized cultures live for 4–5 years. Liquid or agar cultures may persist for 3–5 years. The organisms may live at 37° C. for 2 weeks. Sunlight destroys cells in a few hours but cultures remain viable for 900–1500 days in the dark.

**Staphylococcus (Micrococcus)* (Table C21). Dried cultures have remained viable for 30 years. Stock lab cultures on media sealed remained alive for 11–12 years. At room temperatures, cultures remain alive for 1½–2 years. Lyophilized strains were alive for 4–5 years. Exposure to saline, freezing, vacuum drying, extreme freezing temperatures result in lessened survival.

**Streptococcus* (Table C22). Dried cultures may live over 97 days but if lyophilized, viability of 4–7 years has been reported. Liquid cultures may live 30–60 days, if tissue added then lives 11–12 months. On solid media sealed tube cultures lived 11–12 years. Increased humidity over cultures lowers survival from 3 years to 8 weeks.

**Treponema* (Table C23). When dried under varying conditions of humidity and temperature, the cells were killed in several hours to several days. Survival at freezing temperature in media for several weeks to 2 months. At subfreezing temperatures survival of 1–3 years was obtained when tissue added. Exposure effects of heat, pH changes and freezing temperatures are given.

**Vibrio comma* (Table C24). Dried preparations survived 4 years. Lyophilized preparations were viable for about 4–5 years. Liquid cultures lived for 4–5 weeks. On solid media lived under lab conditions for 6 weeks to 20 weeks. Some agar cultures dried, lived only 2–11 days. At extremely low sub-freezing temperatures, viability for over a year was found.

**Viruses* (Table C25)

Herpes—Dried virus at —5° C. survived over a year; at 37° C. for 2 months. Lyophilized, survival given at 4 weeks only. Liquid preparations at 37° C. for 100 hours and at 42.5° C. for 30 to 80 hours. UV radiation destroyed in 10 minutes. At —70° C. viability of over 1 year was found.

Hoof and Mouth—In dried form survived high temperatures for short periods. Ten day viability at room temperature is listed. In liquid preparations such as blood 2–5 day viability found; in buffered lymph over 2 year viability, high pH and repeated freezing did not destroy in 124 days. In solid media, 162 day survival is reported.

Influenza—Dried in talc for 30 minutes destroyed virus. Lyophilized, viable and infectious for 14 months. At —78° C. viability of 6 months to 3 years is noted in broth plus tissue. At —20° C. less than 6 month survival is recorded. Low pH was found to be detrimental. Temperatures over 40° C. destroyed the virus quickly.

Poliomyelitis—When dry, 52° C. for 30 minutes necessary for destruction. In glycerin, survival of 6 years is reported by one, others report over 2 year survival in glycerin. Concentration of glycerin and storage temperature are important. At 38° C., virus destroyed in 7 days.

Smallpox—Dried and stored at 37° C., 80 day survival but at 4–6° C. only 24 hour survival. In media, viable only 30 minutes at 35° C.

Vaccinia—Dried virus at 4° C. lived 12–18 months; 229 days when dried,

temperature not given; lyophilized, lived for 10 months. In glycerin, at —70° C. 21 month survival, at refrigerator temperature it was avirulent in 12 months. In lymph for 2 years; in allantois at low temperature, for 15 years. Dry lymph in tropics survived for 18 years.

Tobacco Mosaic—When dried it remained active for many years.

Laryngotracheitis—The virus remained active for 3 years following lyophilization and storage at 4° C.

Lymphogranuloma inguinale—Ten month viability has been reported for the virus in rabbit testes in infusion broth at —78° C. At 37° C. viability of 2–4 days reported with 56° C. destroying in 10 minutes and —70° C. allowing viability for over a year.

Lymphocytic choriomeningitis—Frozen dried material at 5° C. remained active for over a year.

Meningopneumonitis—Viability for 3 years in broth plus tissue at —78° C.

Encephalitis, St. Louis—Frozen dried preparations at 5° C. were active for over 833 days. At 40° C. and pH 8.4, 3 week viability was found. Heat at 56° C. for 30 minutes necessary for destruction.

Encephalitis, Jap B—In tissue plus serum at —20° C., 6–12 month survival reported. pH levels above 7 inactivated the virus rapidly, 60–70° C. destroyed it in 10 minutes.

Encephalitis, equine—Acid pH inactivated it readily as did alkaline pH levels.

Enteritis—At pH 7, the virus survived only 20 days.

Measles—At —20 to 30° C., the virus was viable for only 6 months or less. Acid and alkaline pH levels inactivate readily.

Newcastle—In 50 per cent glycerin at pH 7.6, the virus was viable for 95 days at 25° C. and at 5° C. was viable for a year. At 37° C., one report lists 126 days of activity.

Psittacosis—Viability in broth for 29 days at lab temperatures, while at —70° C. for over 2 years active virus was present.

Rabies—The virus is apparently quite stable, surviving 56° C. for an hour, —185° C. for 3 months and living at 25° C. for several weeks. Extraction with ether at —65° C. still gives active virus after 1 year.

Coxsackie—Temperatures of 53–55° C. for 30 minutes inactivate. Acid and alkaline pH levels inactivate in 1 day.

Yellow Fever—Aqueous suspensions are viable for 10 days, with glycerol, 8 months viability is found. Dried and frozen the virus is active for years.

Cowpox—At sub-freezing temperatures survival of 1–4 days is revealed.

Rinderpest—Desiccated virus viable for 15 days. In tissue at 4° C. over 4 month survival.

"Cold" virus—At 4° C., the filtrates are active for 3 days, at 10° C. for 27 days and at —76° C. for 2 years.

•*Yeasts, Molds and Fungi* (Table C26)

Actinomyces—Dried cultures survive from 1–5 years.

Saccharomyces—When dried the cells are quite resistant. Ten month survival in plaster of paris. Lyophilized cultures were viable for 1–2 years. In liquid cultures survival of 5 weeks to over 160 weeks at —15° C. are reported. In 10 per cent sucrose, 8–10 year viability found. On solid media at —70° C., survival of a week is listed. At —10° C. over a year survival is reported while at 37° C. 5 month viability was found. Some grow at refrigerator temperatures.

Aspergillus—At sub-freezing temperatures survival of 4 days was observed, while at 7° C. on agar over 2½ year viability was found. At 25° C. in the dark, 6 year storage was reported for one while others lived 10–16 years.

Epidermophytes—Survived for several weeks at sub-freezing temperatures.

Blastomyces—Over 20 months at 25° C. on agar covered with oil.

Candida—Survival of 20 months at 25° C. on agar covered with oil.

Coccidioides—On agar at 25° C., survival of 20 months when covered with oil.

Cryptococcus—Recovery of 20 months on agar slants covered with oil stored at 25° C.

Nocardia—20 month survival observed.

Streptothrix—420 day survival reported.

Others—Many fungi such as Penicillium, Rhizopus and others survived over 2½ years on agar at 7° C.

TABLE C1

The Survival of Bacillus anthracis in Culture

Factor(s)	Survival	Reference	
SOLID, Continued			
Agar, incub. temp.	Viable 24 hrs.	Sanfelice	1893
Agar cult. 2–3 days old	5% incapable of germinating 7 hrs.	Swann	1924
Agar cult. 8 mos. old, kept over fused $CaCl_2$	7 hrs.	Swann	1924
Gelatin agar, 180 C., sunlight for 6 d.	2–6 hrs.	Ward	1892
GENERAL			
80 C. (Str. R1009)	Inoc. 234, Recov. 200 after 2 hrs. expos. to alkali	Jones	1942
Best time & temp for heating industrial material suspected infected with anthrax 5 min. @ 65 C.		Jones	1942
Continuous freezing at —60 to —70 C., blood susp.	Organisms destroyed in 90–124 d.	Stein	1947
18 hr. serum broth cult. —5 to —10 C.	Viable for G.P. after 10 yrs., 8 mos.	Stein	1947
Boiling	10 min.	Topley	1936

TABLE C2

The Survival of Bacillus Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
B. spp.			
Dried	4–5 yrs.	Proom	1949
LIQUID			
B. cereus			
Broth, before centrifug.	100% survive	Winslow	1927
Peptone, after centrifug.	56% survive	Winslow	1927
1 hr. later	71% survive	Winslow	1927
Peptone, before centrifug.	100% survive	Winslow	1927
Peptone, after centrifug.	43% survive	Winslow	1927
1 hr. later	53% survive	Winslow	1927
Meat extract, before centrifug.	100% survive	Winslow	1927
Meat extract, after centrifug.	51% survive	Winslow	1927
1 hr. later	57% survive	Winslow	1927
½ Locke-Ringers, before cent.	100% survive	Winslow	1927
½ Locke-Ringers, after cent.	3% survive	Winslow	1927
1 hr. later	1% survive	Winslow	1927
B. globigii			
Susp. 98.5 F.	8–16 min.	Davis	1948
Susp. 98.5 F.	Innoc. 100 million spores Recov. 0, 16 min.	Davis	1948
Gentian violet, $HgCl_2$	Resistant to action	Davis	1948
Streptomycin	Resistant to action	Davis	1948
98.5 F.	12 min. (heat resist)	Davis	1948
B. megatherium			
Bouillon cult., direct sunlight on paper slips	Spores, 6 h.	Weinzirl	1914
Preisz-Nocard bacillus			
Serum in flask, light	11 mos.	Urbain	1930
Serum in flask, dark	13 mos.	Urbain	1930
B. radicicola			
Solution	142 d.	Alicante	1926
B. radiobacter			
Solution	10 min.	Alicante	1926
B. subtilis			
High press., 17,600, R.T.	45 min.	Basset	1932
Alcohol and toluol	6 mos.	Hite	1938
Bouillon cult., sunlight	Bact. 8.5 h., spores, 5 h.	Weinzirl	1914
B. vulgatus			
Bouillon cult., sunlight	5 h.	Weinzirl	1914
SOLID			
B. alcaligenes			
Agar, exp. to UV, 30 min.	Acquired slight germicidal properties	Tanner	1930
B. fluorescens putridus			
Agar and gelatin plates exp. to sunlight	2½ hrs.	Dieudonne	1894
B. megatherium			
Agar covered with sterile 10% cane sugar, —10 C.	8 mos.	Keith	1913
Agar, exp. to UV, 30 min.	Acquired slight germicidal properties	Tanner	1930

TABLE C2—(Continued)

The Survival of Bacillus Species in Culture

Factor(s)	Survival	Reference	
SOLID			
B. proteus			
Nutrient agar, 0–5 C.	No growth	Haines	1934
B. subtilis			
Agar covered with sterile cane sugar, —10 C.	8 mos.	Keith	1913
Agar, exp. to UV, 30 min.	Acquired slight germicidal properties	Tanner	1930
GENERAL			
B. megatherium			
—5 to —15 C.	Resisted better than higher temp.	Campbell	1932
	>278 d.	Lal	1921
B. mesentericus			
Broth, —13 to —15 C.	>80 wks.	Tanner	1928
Salt soln., —13 to —15 C.	>80 wks.	Tanner	1928
B. subtilis			
Broth, —13 to —15 C.	>80 wks.	Tanner	1928
Salt soln., —13 to —15 C.	>80 wks.	Tanner	1928
B. globigii, B. thermoacidurans, B. subtilis			
Amino acids and growth factors had little if any effect on sporulation. Heat resistant spores were developed. Resistance to phenol and iodine paralleled heat resistance.		Williams	1948
B. brevis, B. subtilis, B. megatherium, B. globigii, B. cereus			
Species of anaerobic spore forming bacilli that produce spores of unequal heat resistance. Spores of B. cereus were not affected by any of the amino acids, growth factors, or yeast nucleic acids as nutrients.		Williams	1951
B. mycoides			
Bouillon cult., direct sun on paper slips	Bacteria 6 hrs., spores 5 hrs.	Weinzirl	1914
Bacterium linens			
Litmus milk, 20 C.	Inoc. 132,000,000/ml. 1/ml., 17 d.	Albert	1944
Litmus milk, R.T.	4 mos.	Albert	1944

TABLE C3

The Survival of Bacteriophage in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
Typhoid, phage			
32% NaCl dehydrated in Raust-Heim apparat.	Viable 26 yrs.	Marcuse	1949
Phage, general			
Dried in vacuo	3½ yrs.	Campbell	1941
Dried	More resistant to heat	Vedder	1932

TABLE C3—(Continued)

The Survival of Bacteriophage in Culture

Factor(s)	Survival	Reference	
DRIED, LYOPHILIZED			
E. coli and staphylococci			
—78 C. and thawed 20X	Did not lose activity	Sanderson	1925
1st thawing	16% failed to survive	Sanderson	1925
10th thawing	86% failed to survive	Sanderson	1925
16th thawing	99% failed to survive	Sanderson	1925
Dysentery phage			
Addition of 1% gelatin with lyophilized activity of dysentery phage. Raw egg whites protected phage for inactivating effect of glycogen. Difco yeast, difco brain-heart infusion, fresh aqueous extracts of heart, muscle, liver, pancreas, brain, thymus, and kidney effective in promoting stability of sensitive phage during lyophilization. Lecithin extracted from raw egg yolk gave protection to sensitive phage equal to that afforded by egg yolk itself. Stable on exposure for 48 h. to temp. of 55 C. Passage through 0.5% HC_1 causes loss of lytic activity. Loss of activity followed desiccation. No particular pH favors lytic activity. Addition of cysteine, ascorbic acid and removal of O_2 prior to lyophilization failed to effect stability.		Schade	1944
GENERAL			
Staphylococcus phage			
.01–1% methylene blue, indirect sunlight	5 min.	Clifton	1931
E. coli phage			
2–5 C. (B1)	11 yrs.	Raketen	1947
2–5 C. (B1 and B2)	7–17 yrs.	Raketen	1947
Dysentery phage			
Powdered and kept dry, 37 C.	No loss in activity after 6 mos.	Schade	1943
Addition of meat, gelatin, dried egg alb., peptone, gastric mucin, human serum and human plasma	Non constant protection for phage	Schade	1944
pH 3.3–4.3, 48 hrs., 18 C.	Survives	Sierakowski	1930
Less resistant to same pH at 37 C. in acid med.		Sierakowski	1930
pH 10–9.55, 24 hrs., 18 C.	Survives	Sierakowski	1930
More resistant to same pH levels at 37 C.		Sierakowski	1930
More resistant to pH than corresponding organism		Sierakowski	1930

TABLE C4

The Survival of Borrelia, Leptospira and Spirillum in Culture

Factor(s)	Survival	Reference	
DRIED, LYOPHILIZED			
Borrelia			
—78 C. (duttoni)	1 yr.	Turner	1939
Blood drawn early in disease		Lofgren	1945

TABLE C4—(Continued)

The Survival of Borrelia, Leptospira and Spirillum in Culture

Factor(s)	Survival	Reference	
DRIED, LYOPHILIZED, Continued			
regains motility better than blood drawn late. (novyi)			
—78 C. (novyi)	1 yr.	Turner	1939
Rat blood, —12 to —20 C. (recurrentis)	Infectivity reduced 6 wks.	Turner	1939
Warming from —78 C. to 0 C. over 2–6 hr. period (swellengrebel)	Kills most of organisms	Turner	1939
—78 C. (tickmouse)	1 yr.	Turner	1939
Dried from frozen state (vincenti)	192 hrs.	Hampp	1947
Lyophil. vaccine has greater stability than regular liquid vaccine		Verwey	1950
Leptospira icterohemorrhagiae			
Rabbit testes in infusion broth, —78 C.	10 mos.	Turner	1939
20% tissue susp. in infusion broth, —78 C.	Actively motile 10 mos.	Turner	1939
Spirillum minus			
Mouse blood and peritoneal fluid, —78 C.	Viable for mice, 1 yr.	Turner	1939
LIQUID			
Leptospira icterohemorrhagiae			
Serum Ringers soln., 42 C.	5 d.	Anjow	1928
Physiol. saline, 29 C.	20 d.	Reitano	1939
Physiol. saline, variable temp.	8 mos.	Reitano	1939
Serum, 37 C.	6 wks.	Reitano	1939
Serum, 30 C.	3 mos.	Reitano	1939
Serum, variable	7 mos.	Reitano	1939
.2% guinea pig blood, 30 C.	16 mos.	Reitano	1939

TABLE C5

The Survival of Brucella Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
Brucella, species			
Dried	4–5 yrs.	Proom	1949
LIQUID			
Brucella abortus			
Broth, 15–20 F., dry	400 d.	Feldman	1935
Saline, 62 C.	All dead 1 min.	Seelemann	1938
Brucella melitensis			
Broth	15 d.	Bellelli	1928

TABLE C5—(Continued)

The Survival of Brucella Species in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Brucella suis			
Aerated broth cult. with dextrose and ascorbic acid 20–25 C.	2 mos.	Elberg	1947
Brucella, species			
Soln. buffered to pH 7.2	4–5 wks.	Zobell	1932
SOLID			
Brucella melitensis			
Nutrient gel, 22 C.	No change 8 d.	Ransome	1901
GENERAL			
Brucella abortus			
Incub. 30 d. @ 37 C.	Smooth replaced by Smooth X at end of 30 d.	Goodlow	1951
The three smooth types were similar in virulence, antigenic prop. The three differed in resistance to alanine which accumulates in culture fluid. Original smooth type will grow in alanine.		Goodlow	1951
Elevation in temp. causes reduction in number of viable cells. Recovery when difference between tmp. of ice film and surface of surrounding sample is small. At high moisture per cent of viable cells is very low. Increase in dryness increases survival after storage. Slow rate of drying increases per cent viable cells. Recovery of viable cells after freezing-drying low unless NaCl present		Hutton	1951
2–5 C.	Innoc. 25 b/cc. Recov. 20.2 b/cc., 100 d.	Verwey	1950
Optimum pH 7–7.4		Zobell	1932
Brucella melitensis			
Stored 37 C.	17.3% survival 4 yrs.	Stamp	1947
Brucella, species			
Sunlight and drying	Lowers incidence of disease	Polding	1947

TABLE C6

The Survival of Clostridium Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
Cl. novyi	4–5 yrs.	Proom	1949
Cl. perfringens	3–4 yrs.	Proom	1949
Cl. septicum	3–4 yrs.	Proom	1949
Cl. spp.	4–5 yrs.	Proom	1949
DRIED, LYOPHILIZED			
Cl. botulinum			
Sorenson buffer soln. pH 6.9, frozen with carbon dioxide	±20 C. for days had no effect on spores, slow or quick freezing had no effect on spores	James	1933

TABLE C6—(Continued)

The Survival of Clostridium Species in Culture

Factor(s)	Survival	Reference	
LIQUID			
Cl. tetani			
Glycerine under high pressure (13,500 atm)	20% active 45 min.	Basset	1932
Cl. botulinum			
Uninocculated dextrose broth exposed to O_2 for 1 hr.	Recov. 32–81%	Dack	1929
Uninocculated dextrose broth exposed to O_2 for 1 hr. pH 6.8	Recov. 0–57%	Dack	1929
Unbuffered salt soln.	Recov. 0–37%	Dack	1929
Subcult. in beef heart 100 C. with 4% peptone	Recov. in 1½–2½ hrs.	Sommer	1930
Above plus PO_4	Recov. in 4 hrs.	Sommer	1930
Casein digest	Heat resist. 4½ hrs. Most resistant in 4–8 day cultures	Sommer	1930
10 C. refrig. foods	Growth only after 27 d.	Tanner	1940
5 C. refrig. foods	No growth up to 108 d.	Tanner	1940
Glucose, beef infusion broth, heated in water bath 70–73 C.	10 min.	Thom	1919
10% salt broth, R.T., Lg. number strains ranging from 1–10 not inhibited when in alkaline med.	>49 d.	Wyant	1920
Cl. tetani			
Grown in dextrose bouillon 3 mos.	Viable 2½ mos.	Wesbrook	1896
SOLID			
Cl. tetani			
Sealed agar tubes	38 yrs.	Boventer	1947
Agar, 37 C.	20–25 d.	San Felice	1893
GENERAL			
Cl. botulinum			
>5 F.	140 d.	Campbell	1932
100 C., moist, 5 hrs. pH 6.8–7.0	Spores destroyed	Tanner	1923
105 C., moist, 2 hrs., pH 6.8–7.0	Spores destroyed	Tanner	1923
110 C., 1.5 h., pH 6.8–7.0	Spores destroyed	Tanner	1923
115 C., 40 min., pH 6.8–7.0	Spores destroyed	Tanner	1923
120 C., 10 min., pH 6.8–7.0	Spores destroyed	Tanner	1923
16 C.	Young spores more resis. 14 mos.	Tanner	1931
16 C., in fruits and veg.	>2 yrs.	Tanner	1931
Direct sunlight, cotton plugged tubes	24–40 h.	Thom	1919
Diffuse light, cotton plugged tubes	2 mos.	Thom	1919
100 C.	1 h.	Thom	1919
100 C., pH 7.5	Killed 5 h.	Weiss	1921
105 C., pH 7.5	40 min.	Weiss	1921
120 C., pH 7.5	6 min.	Weiss	1921

TABLE C6—(Continued)

The Survival of Clostridium Species in Culture

Factor(s)	Survival	Reference	
GENERAL, Continued			
	Decrease in thermal resistance as spores age. H ion decreases resistance of spores to heat.		
Cl. fragilis			
37 C.	24–30 h.	Repaci	1910
Refrig.	1 mo.	Repaci	1910
Cl. moniliformis			
37 C.	4 wks.	Repaci	1910
R.T.	2½ mos.	Repaci	1910
Cl. perfringens			
37 C.	3 d.	Repaci	1910
Refrig.	1 mo.	Repaci	1910
Cl. tethoide			
37 C.	6 d.	Repaci	1910
R.T.	6 wks.	Repaci	1910

TABLE C7

The Survival of Corynebacterium Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
C. diphtheriae			
Dried	4–5 yrs.	Proom	1949
C. Magnusson-Holth			
Dried, dark	6 wks.	Ottosen	1945
Daylight	2–4 wks.	Ottosen	1945
C. pyogenes	4–5 yrs.	Proom	1949
C. spp.			
Dried	4–5 yrs.	Proom	1949
LIQUID			
C. diphtheriae			
Bouillon	Recov. 0, 24 h.	Abel	1893
Washed serum, dried in daylight	121 d.	Abel	1893
Susp. in physiol. sal. soln., washed —48 C.	6 mos.	Cordova	1949
2% glycerine, —48 C.	6 mos.	Cordova	1949
Phosphate buffer, —48 C.	6 mos.	Cordova	1949
Proteose peptone, —48 C.	6 mos.	Cordova	1949
Old bouillon	1 cult., viab. 6 mos.	Kasansky	1899
Bouillon susp. from diph. memb., R.T.	Innoc. 100,000 col., recov. 0, 21 h.	Kirstein	1902
Bouillon susp., from diph. memb., in daylight	24–48 h.	Kirstein	1902
Bouillon susp., R.T., cellar	5 d.	Kirstein	1902
Bouillon	523 d. (Bombay Str.)	Lal	1921
Broth emuls., unster. milk, liquid air	No impaired vitality 7 d.	MacFayden	1900

TABLE C7—(Continued)

The Survival of Corynebacterium Species in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Liquid hydrogen, —252 C.	10 h.	MacFayden	1900
Physiol. saline, R.T., absolute drying over sulfuric acid in vacuo	Innoc. blood or serum agar. Viable for mos.	Otten	1930
Sensit. with Met. violet	Recov. 0, 5 min.	Philibert	1926
Pure culture swabs, dark	60 d. Pathogenic 40 h. following isolation Pathogenic 50 hrs. following desic. with light	Schofield	1916
Liquid air in glass	Recov. 35% in 2 hrs.	White	1901
C. Magnusson-Holth			
.5% formaldehyde	Killed in 3 h.	Ottosen	1945
C. pyogenes			
Pure culture	30 d.	Kirstein	1902
SOLID			
C. diphtheriae			
Gelatine	18 mos. still alive	Abel	1893
Gelatine in dark	331 d.	Abel	1893
Agar cult.	7 mos.	Abel	1893
Agar cult. in dark, R.T.	Still viable 171–172 d.	Abel	1893
Loefflers, —23.5 C., old cult., 24 d.	Viable 86 d.	Abel	1895
Loefflers, —23.5 C., old cult., 24 d.	Viable 86 d.	Abel	1895
Loefflers, —23.5 C., old cult., 24 d.	Str. 2, no growth 86 d.	Abel	1895
Loefflers, —23.5 C., old cult., 24 d.	Str. 3, 2 col. viable 86 d.	Abel	1895
Glycerine agar tubes 37 C., ozonized air aspirated through tube	No change at end of 8 d.	Ransome	1901
GENERAL			
C. diphtheriae			
Incubator, dried, R.T.	7 mos.	Abel	1893
DRIED			
erysipelothrix rhusiopathiae			
Dried	4–5 yrs.	Proom	1949
Stored at 37 C.	2.6% survived 4 yrs.	Stamp	1947

TABLE C8

The Survival of Diplococcus Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
D. pneumoniae			
Dried and formalized with modified gastric mucin	9 weeks, agglutinated 4 weeks, capsules disintegrated	Bourn	1947

TABLE C8—(Continued)

The Survival of Diplococcus Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL, Continued			
Dried	7–8 yrs.	Lal Kalra	1948
38 C.	6–8 yrs.	Patella	1888
Less than 17 C.	4–5 yrs.	Proom	1949
DRIED, LYOPHILIZED			
D. pneumoniae			
Dried, frozen, plain broth	3 yrs.	Stillman	1941
Dried, frozen, plain broth	Type 1, recov. 42% 3 yrs.	Stillman	1941
Dried, frozen, plain broth	Type 2, recov. 69% 3 yrs.	Stillman	1941
Dried, frozen, plain broth	Type 3, recov. 62% 3 yrs.	Stillman	1941
Dried, frozen, plain broth	Type 8, recov. 72% 3 yrs.	Stillman	1941
Dextrose agar	>3 mos.	Swift	1921
LIQUID			
D. pneumoniae			
Broth, saliva or 0.5% saline susp. RH 50%	High mortality	Dunklin	1948
Physiol. saline, R.T., abs. drying in vacuo	Innoc. on blood or serum agar. Viable mos.	Otten	1930
Sensit. with Met. violet	Recov. 0–15 min.	Philibert	1926
36–38 C. with Hb.	50–60 d.	Rymowitsch	1902
Beef bouillon	36 h.	Rymowitsch	1902
"T" med. with 2 vol. 15% gel. in phys. saline, ice-box temp.	>6 mos	Truche	1912
Blood with glucose or serum broth, ice box, hermetically sealed	Several mos.	Yourevitch	1930
SOLID			
D. pneumoniae			
1 part nutrient agar and 5 parts sterile ascitic or pleuritic fluid	Short incubation >3 mos.	Wadsworth	1903
Stock agar cult. covered with thin layer rabbit blood	50 d.	Washbourn	1896
GENERAL			
D. pneumoniae			
37 C.	>132 d.	Lal	1921
Change in pH from 7.80 to 4.47 from 13 h. to 1 h.	Progressive increase in death rate	Lord	1922
80 F., daylight	Type 3, Smooth 5 mos.	Stillman	1940
110 C., dry	30–60 min.	Yesair, J.	1946
56 C., moist	<60 min.	Yesair, J.	1946

TABLE C9

The Survival of Escherichia Coli in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
Dried in desiccator	>10 d.	McNaught	1910
Dried	4–5 yrs.	Proom	1949

TABLE C9—(Continued)

The Survival of Escherichia Coli in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL, Continued			
Vacuum dried, 195 C.	4 d.	Shattock	1912
Air dried, dark	4 d.	Shattock	1912
DRIED, LYOPHIL			
Susp. in broth, —185 C.	Innoc. 180,000,000	Rivers, T.	1927
Frozen and thawed 12 times	Recov. 40,000	Rivers, T.	1927
LIQUID			
Saline susp. 6–11 C., RH 70–85%, CO_2 5–4%	>5 d.	Arai	1931
1% glucose or lactose in ½% Witte peptone and ½% dipotassium in dist. water	Innoc. 100,000,000 after 24 hrs. recov. 0, 5 d.	Burling	1918
Dextrose bouillon. Freezing vacuum desic., freeze 47 h. Culture age 48 hrs.	>19 d.	Hammer	1911
Above with culture age 24 hrs. freezing 24 hrs.	57 d.	Hammer	1911
Bouillon culture, 53 C.	Innoc. 2,200,000,000/cc. Recov. 190,000,000/cc in 62 h. Innoc. 15,000,000/cc Recov. 1500/cc, 1¾ h. Innoc. 14,500,000/cc Recov. 7,000/cc, 2¼ h.	Heiberg	1932
Glucose soln. —6 C.	50% dead	Hilliard	1918
Glucose, tap water, —10 C.	3 hrs.	Hilliard	1918
Glucose soln. —0.5 C.	3 hrs.	Hilliard	1918
Glucose soln. —1.5 C.	3 hrs.	Hilliard	1918
Glucose soln. —6 C.	3 hrs.	Hilliard	1918
M/15 PO_4 buffer, 2 C., irrad., O_2 tension lowered	Rapid destruction of 90% or more when frozen in tap water for 3 hrs.		
	Showed ⅓ sensitivity when oxygen tension lowered by saturating with N_2 or CO_2. Protect. given by adding glucose or addition of cysteine	Hollander	1951
Sterile buffer soln.	Kill 80%, 10 min.	Horwood	1950
15.5 C.	Kill 90%, 15 min.	Horwood	1950
With ultra sonorator	Kill 99%, 40 min.	Horwood	1950
1% soap soln., 4–8 C.	6 h.	Jolle	1895
2% soap soln.,4–8 C.	6 h.	Jolle	1895
8% soap soln., 18 and 30 C.	30 min.	Jolle	1895
6% soap soln., 18 and 30 C.	1 h.	Jolle	1895
3% soap soln., 18 and 30 C.	6 h.	Jolle	1895
1% soap soln., 18 and 30 C.	24 h.	Jolle	1895
Buffered media, 5 C.	Maximal survival	Jordan	1948
pH 6.5–6.6	610 min., 90% mortality	Jordan	1948
pH 7.0–6.4	abnormally sensitive	Jordan	1948
pH 2.8	99% mortality, 15.6 ∓ .12 min.	Jordan	1948

TABLE C9—(Continued)

The Survival of Escherichia Coli in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
.85% NaCl, —252 C.	3 h.	Kadisch	1931
NaCl soln.	Still recoverable 6 wks.	Karaffa	1912
5–42% C.P. glycerine, —20 C. susp. in water	Large per cent alive >6 mos.	Keith	1913
Cultures, R.T., dark, in inverted Pasteus dish	358 d.	MacConkey	1905
—185 C. liquid air	No impaired vitality 20 h.	MacFayden	1899
Broth emulsion with ster. milk, liquid air	No impaired vitality 7 d.	MacFayden	1900
Quill tubes, liquid air	6 mos.	MacFayden	1900
Liquid H_2, —252 C., sealed	10 h.	MacFayden	1900
Lactose peptone water	5–18 d.	Oshii	1920
Serum, 37 C.	30 d.	Panisset	1925
Sensit. with met. violet	Recov. 0, 2 h.	Philibert	1926
Saline, —78 C., freeze in CO_2 ice	21.5%	Proom	1949
Broth, —78 C., freeze in CO_2 ice	52%	Proom	1949
Broth, R.T.	43.3%	Proom	1949
Saline, —78 C.	64.6%	Proom	1949
Ringers soln., pH 2	Recov. 1%, 4 h.	Shaugnessy	1925
Ringers soln., pH 6	Recov. 198%, 4 h.	Shaugnessy	1925
Ringers soln., pH 8	Recov. 65%, 4 h.	Shaugnessy	1925
Ringers soln., pH 11	Recov. 0%, 4 h.	Shaugnessy	1925
NaCl soln., 1.45 M, pH 2	Recov. 2%, 4 h.	Shaugnessy	1925
NaCl soln., 1.45 M, pH 6	Recov. 65%, 4 h.	Shaugnessy	1925
NaCl soln., 1.45 M, pH 8	Recov. 0%, 4 h.	Shaugnessy	1925
NaCl soln., 1.45 M, pH 11	Recov. 0%, 4 h.	Shaugnessy	1925
NaCl soln., .725 M, pH 2	Recov. 0%, 4 h.	Shaugnessy	1925
NaCl soln., .725 M, pH 6	Recov. 41%, 4 h.	Shaugnessy	1925
NaCl soln., .725 M, pH 8	Recov. 4%, 4 h.	Shaugnessy	1925
NaCl soln., .725 M, pH 11	Recov. 0%, 4 h.	Shaugnessy	1925
NaCl soln., .0145 M, pH 2	Recov. .1%, 4 h.	Shaugnessy	1925
NaCl soln., .0145 M, pH 6	Recov. 88%, 4 h.	Shaugnessy	1925
NaCl soln., .0145 M, pH 8	Recov. 60%, 4 h.	Shaugnessy	1925
NaCl soln., .0145 M, pH 11	Recov. 0%, 4 h.	Shaugnessy	1925
CaCl soln., 1.45 M, pH 2	Recov. 0%, 4 h.	Shaugnessy	1925
CaCl soln., 1.45 M, pH 6	Recov. 0%, 4 h.	Shaugnessy	1925
CaCl soln., 1.45 M, pH 8	Recov. 0%, 4 h.	Shaugnessy	1925
CaCl soln., 1.45 M, pH 11	Recov. 0%, 4 h.	Shaugnessy	1925
Balanced salt soln., may be distinctly favorable to bact. viab. in water at optimum react., while distinctly unfav. in slightly more alk. soln. Acid soln. kills rapidly. Neutral pH favors viability			
NaCl soln.	Buffering capacities reduced with all strengths used	Shaugnessy	1924
$CaCl_2$ soln.	Buffering capacities partial abolished in all solns. which were nontoxic at favorable pH	Shaugnessy	1924

TABLE C9—(Continued)

The Survival of Escherichia Coli in Culture

The Survival of Escherichia coli in Culture			
LIQUID, Continued			
NaCl and $CaCl_2$	Showed no material inc. in buffering capacities over that of the soln. cont. $CaCl_2$ alone	Shaugnessy	1924
NaCl soln.	Still recoverable 6 wks.	Stadler	1899
Broth, —13 to —15 C.	Recov. .1%, 8 wks.	Tanner	1928
Salt soln, —13 to —15 C.	Recov. .1%, 12–16 wks.	Tanner	1928
10% sucrose susp., —195 C.	Innoc. 6,000,000/ml. 48.6% reduct., 2½ h.	Weiser	1946
10% sucrose susp., denitrified, —195 C.	Innoc. 6,000,000/ml. 55.1% reduct., 2½ h.	Weiser	1946
10% sucrose susp., crystallized, —195 C.	Innoc. 10,000,000/ml. 33.0% reduct., 2 h.	Weiser	1946
Peptone buffer mix., —195 C. pH 7.0, susp., 20 h. cult.	Death slow and difficult to detect, 10 h.	Weiser	1945
Broth, before centrifug.	100% survival	Winslow	1927
Broth, after centrifug.	70% survival	Winslow	1927
Broth, 1 h. after centrifug.	70% survival	Winslow	1927
Broth cult. mixed with sea sand, 69 C., RH 90%	8 h.	Winslow	1911
Above with RH 72%	10 h.	Winslow	1911
Above, temp. 70 C., RH 60%	7 h.	Winslow	1911
Above, temp. 69 C., RH 90%	8 h.	Winslow	1911
SOLID			
Raw milk on 1% lactose agar and added to litmus milk, incub. 15 d. @ 20 C.	Generation time lengthened	Allen	1923
Agar and gelatin plates, exposed to sunlight, Nov.	1½ h. (March, July, Aug.) 2½ h. (Nov.) 4½ h. (Winter)	Dieudonne	1894
Salt media	91 d.	Frank	1941
Nutrient agar, R.T., stored in dark	Many viable cells 14 yrs.	Sears	1946
Agar exposed to U.V. 30 min.	Acquired slight germicidal power	Tanner	1930
Agar exposed to X-ray	C-9 mutant strains produced	Tatum	1945
Nutrient agar with Na, 37 C.	Less toxic than Ca. mixt. of Ca and Na less toxic and Bact. inc.	Winslow	1928
$CaCl_2$ 0.00145 M	Recov. 152%, 24 h.	Winslow	1923
NaCl 0.0145 M	Innoc. 100%, Recov. 140%, 24 h.	Winslow	1923
Gelatin 20 C., 1% acid add to phenolphthalein	>11 mos.	Worth	1919
GENERAL			
Cells of E. coli were found to remain dormant for 16 d., 85% develop in 48 h.		Burke	1925
Heat shocked 30 min. 53 C. temp.	Innoc. 1,467,000, Recov. 120,000, 38 hrs.	Elliker	1938

TABLE C9—(Continued)

The Survival of Escherichia coli in Culture

Factor(s)	Survival	Reference	
GENERAL, Continued			
Heat shocked 30 min. 28 C. temp.	Innoc. 1,674,000, Recov. 570,000, 37 hrs.	Elliker	1938
Heat shocked 30 min. 30.5 C. temp.	Innoc. 1,510,000, Recov. 427,000, 42 hrs.	Elliker	1938
Heat shocked 30 min. 38.5 C. temp.	Innoc. 1,341,000, Recov. 875,000, 20 hrs.	Elliker	1938
Heat shocked 30 min. 40 C. temp.	Innoc. 1,413,000, Recov. 267,000, 10 hrs.	Elliker	1938
Culture med. (1½–3 yrs. old) 10 C. following growth at 45 C.	95% reduction in 1 h. due to temp. change	Hegarty	1940
Susp. E. coli, hem. strep., and acid fast saprophytes heated various lengths of time. Inc. in counts greatest in plates heated longest. Greatest inc. in E. coli and strep. 24–48 h.		Isaacs	1930
Growth in culture	1,562 d.	Lal	1925
Desiccated	Death proceeds logarithmically	Heller	1941
In fluid state	Viable for longer periods than when desic.	Heller	1941
Dist. water, saline, salicin, tryptophane, glucose, xylose, sucrose	Per cent reduction in desic. microorganisms changes from 83.8% to 8.0% in 1 d. With similar cpds. in fluid control, the per cent reduct. changed from 33% to 6.0%	Heller	1941
Variation of resistance in relation to age of cult.	Ratio drops in 2 hrs. then climbs and reaches maximum of 1 at 8 hrs.	Regnier	1938
60 C.	30 min.	Ruska	1941
100 C., broth, dist. water, dried on collodion film.	Viable 1 h.	Ruska	1941
62 C., watery medium	1 h.	Ruska	1941
5–7 C.	24 h.	Ruska	1941
Liquid air, —183 C.	.....	Ruska	1941
—78 C., thawed 20 times	Did not lose activity	Sanderson	1925
45 C., changes suddenly to 10 C.	95% reduction, 1 h.		
	Not affected when changes in temp. take place gradually	Sherman	1934
Young cells, 1 C.	Innoc. 8,600,000, Recov. 0, 42 d.	Sherman	1942
Mature cells, 1 C.	Innoc. 650,000,000, Recov. 10,400,000, >62 d.	Sherman	1942
Stored at 37 C.	12% survive 4 yrs.	Stamp	1947
8 C. rise in temp. at a given conc. NaOH	K inc. by 35–109%	Watkins	1932

TABLE C9—(Continued)

The Survival of Escherichia coli in Culture

Factor(s)	Survival	Reference	
GENERAL, Continued			
Double conc. of NaOH	Increases K 10 times	Watkins	1932
Lime treatment, pH 9–9.5	>540 min.	Wattle	1943
Lime treatment, pH 9.5–10.0	>600 min.	Wattle	1943
Lime treatment, pH 10.0–10.5	>600 min.	Wattle	1943
Lime treatment, pH 10.5–11	>600 min.	Wattle	1943
Lime treatment, pH 11–11.5	>300 min.	Wattle	1943
Rate of storage death at the higher freezing temp. is very rapid and is much greater at the temps. above —30 C. than —30 C. and below. Repeated freezing more lethal than simple freezing or storage in frozen state for similar intervals of time. Freezing much more lethal than supercooling. Repeated fluctuations of temp. of frozen susp. between —30 C. and —78 C. appear to result in lower mortality than storage at either temp. but this protective effect not noted at temp. ranges above —30 C. nor below —78 C. Immediate death occurs at a brief stage in freezing process during which extracellular ice formation is being completed. Mortality due to immediate death does not vary with intensity and freezing time. Rate of storage death at higher freezing temps. is rapid at temps. above 30 C. than less than 30 C. Repeated freezing is more lethal than single freezing or storage in frozen state for intervals of time.			
Stored at —1.5 C.	99.3% reduct. 180–210 min.	Weiser	1945
Stored at —15 C.	85.2% reduct. 120–225 min.	Weiser	1945
Stored at —30 C.	55.3% reduct. 225–270 min.	Weiser	1945
Stored at —78 C.	47.6% reduct. 270–315 min.	Weiser	1945
Stored at —195 C.	39.7% reduct. 285–330 min.	Weiser	1945
Fluctuated at —1.5 to —15 C. 35 times	98.4% reduct. 180 min.	Weiser	1945
Fluctuated at —15 to —30 C. 44 times	57.0% reduct. 220 min.	Weiser	1945
Fluctuated at —78 to —195 C. 46 times	50.8% reduct. 220 min.	Weiser	1945
Acid action on microorganisms is specific		Yaoi	1924
—78 C., thawed 20 times	Did not lose activity	Sanderson	1925

TABLE C10

The Survival of Microorganisms in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
Alcaligenes, spp.			
Dried	4–5 yrs.	Proom	1949

TABLE C10—(Continued)

The Survival of Microorganisms in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL, Continued			
Aerobacter, spp.			
Dried	4–5 yrs.	Proom	1949
Hemophilus			
Dried (spp.)	4–5 yrs.	Proom	1949
Dried (pertussis)	4–5 yrs.	Proom	1949
Klebsiella			
Drying	Months	Loewenberg	1894
Dried	4–5 yrs.	Proom	1949
Lactobacillus			
Dried (spp.)	3–4 yrs.	Proom	1949
Dried 30–37 C. (bulgaricus)	Became inactive in short time	Rogers	1914
Proteus spp.			
Dried	4–5 yrs.	Proom	1949
Pseudomonas			
Dried (spp.)	4–5 yrs.	Proom	1949
Dried in vacuo (pyocyaneus)	Still alive 4 mos.	Shattock	1911
DRIED, LYOPHILIZED			
Lactobacillus bulgaricus			
10 cc. milk, freeze-dry in vacuo, 3–4 hrs.	90–110%	Rogers	1914
Pseudomonas aeruginosa			
Frozen,—24 C., thawed repeatedly at 25 C.	35.6% mortality 1st freezing	Stille	1943
LIQUID			
Aerobacter aerogenes			
Peptone and water susp. spray dried	Recov. 1%	Bullock	1947
Free dried, peptone and water susp.	Recov. 10%	Bullock	1947
Flavobacterium spp.			
—5 to 15 F.	Recov. 0, 77 d.	Campbell	1932
Hemophilus influenza			
Blood broth, —15 C.	2½ h.	Ononato	1902
Blood broth, —20 C.	1½ h.	Ononato	1902
Klebsiella			
Serum, 37 C. (Friedlanders)	43 d.	Panisset	1925
Lactobacillus			
Broth emulsion with unster. milk, liquid air, (acidophilus)	7 d.	MacFayden	1900
Liquid H_2, —252 C.	10 h.	MacFayden	1900
NaCl susp., —10 to —80 C. (acidophilus)	Undiminished count	Mejlbo	1941
.85% NaCl (acidophilus)	Decreased little in 3 d.	Zeug	1920
Milk and lactose, 40–43 C. (bulgaricus)	Innoc. 1 X 10^9/ml. Recov. 100 X 10^9/gm.	Rogers	1914
Lactobacillus casei			
Nutrient broth	Rapid freezing caused less injury than slow freezing	Lund	.....

TABLE C10—(Continued)

The Survival of Microorganisms in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Proteus vulgaris			
NaCl soln.	3 wks.	Karaffe	1912
Liquid air, —185 C.	No impaired vitality 20 h.	MacFayden	1899
Broth emulsion with unster. milk, liquid air	No impaired vitality 7 d.	MacFayden	1900
Liquid H_2, —252 C.	10 h.	MacFayden	1900
Pseudomonas pyocyaneus			
24 h. peptone water cult. vacuum dried	7 mos. and 7 d.	Shattock	1912
24 h. peptone water cult. air dried	4–6 d.	Shattock	1912
SOLID			
Achromobacter, spp.			
Nutrient agar, 0 C.	Rapid growth, 5 d.	Haines	1934
Aerobacter spp.			
24 h. agar cult. in dist. water on filter paper in dry incubator, 37 C.	Alive 31 d.	Hastings	.....
Above with milk cult.	Alive 96 d.	Hastings	.....
Dry corn starch, R.T.	3½ yrs.	Hastings	.....
Azotobacter chroococoum			
Dextrine agar, dried	10 yrs.	Omeliansky	1926
Hemophilus pertussis			
Agar, 20 C.	8 mos.	Worth	1919
Lactobacillus bulgaricus	>657 d.	Lal	1921
Malleomyces mallei			
Glycerine agar tubes, 37 C. ozonized air aspir. through tubes	No change at end of 10 d.	Ransome	1901
Proteus vulgaris			
Agar, 10% sterile can sugar soln., —10 C.	8 mos.	Keith	1913
Pseudomonas			
Agar, covered with 10 T sterile cane sugar, —10 C. (fluorescens)	8 mos.	Keith	1913
Agar, full radiation of Hg. arc. (pyogenes)	1 h. at 5 cm., 15 min. at 8 cm.	Fazzoni	1914
Nutrient agar, 0 C. (spp.)	Rapid growth, 5 d.	Haines	1934
GENERAL			
Aerobacter aerogenes			
Excess lime, pH 9.5–10	>6 min.	Wattle	1943
Excess lime, pH 10–10.5	>600 min.	Wattle	1943
Excess lime, pH 10.5–11	>540 min.	Wattle	1943
Excess lime, pH 11–11.5	>600 min.	Wattle	1943
Erwinia amylonora			
Exudate, 16 C., RH 0–45%	22 d.	Rosen	1938

TABLE C10—(Continued)

The Survival o Mficroorganisms in Culture

Factor(s)	Survival	Reference	
GENERAL, Continued			
Erwinia			
Combinations of moderate temp. and moderate or high humidities proved conducive to short life. Equally high temp. with low R.H. made for long life when organisms in exudate.		Rosen	1938
Hemophilus influenzae			
Blood agar slope, 37 C.	>132 d.	Lal	1921
Physiol. NaCl soln., R.T. sealed tubes. Aboslute drying over sulfuric acid in vacuo	Viable for mos.	Otten	1930
Klebsiella			
Stock lab. cult., R.T., sealed tubes	12–13 yrs.	Ahuja	1935
Malleomyces mallei			
Vacuum, 1–4 C.	25 mos.	Velu	1942
Proteus spp.			
Stock lab. cult., R.T., sealed	19 yrs.	Ahuja	1935
14–30 C.	103 d.	Hilliard	1918
(P. morgagnii)	1,562 d.	Lal	1925
None given	1,562 d.	Lal	1925
Pseudomonas			
(Aeruginosa) freezing 50 h. cult. age 24 h.	17 d.	Hammer	1911
(Pyocyaneous) excluded from light	3 mos.	Shattock	1911
Excess lime, pH 9.5–10	>420 min.	Wattle	1943
Excess lime, pH 10–10.5	>300 min.	Wattle	1943
Excess lime, pH 10.5–11	>240 min.	Wattle	1943
Excess lime, pH 11–11.5	>120 min.	Wattle	1943

TABLE C11

The Survival of Microorganisms in Culture (General)

Factor(s)	Survival	Reference	
LIQUID			
Broth or dist. water, —16 C.	Shorter than in sea water	Hess	1934
—185 C.	No impairment of vitality, 20 h.	MacFayden	1899
Horse serum, vacuum over phosphorus pentoxide	83% viable 14 yrs.	Rhodes	1950
SOLID			
Nutrient agar, 22 C., pH 7.2	103 colonies	Brown	1930
Nutrient agar slants, 37 C. or 30 C. with 572–25, 320 R. X-ray	Order of decreasing resistance: Staph. aureus, E. coli, A. aerogenes, S. marcescens, Ps. aeruginosa, P. fluorescens	Fram	1950

TABLE C11—(Continued)

The Survival of Microorganisms in Culture (General)

Factor(s)	Survival	Reference	
SOLID, Continued			
Greatest in resistance observed with readily absorbed cations		Harris	1949
Petroff medium, bottles evacuated, left in ice box	11 mos. (acid fast)	Harris	1933
GENERAL			
Spring temp.	>40 d.	Duclaux	1885
Summer temp.	>20 d.	Duclaux	1885
Dried organisms more resistant to high temp.		Flosdorf	1938
Slow freezing allows conc. of salts and protein deactivation		Greaves	1944
Destruction of microbial forms at —10 C. faster than at —20 C. compares favorably with other reports.		Haines	1934
Resistance to U.V. light lower in non-pigmented microorganisms than in those producing pigment but not excreting it into medium. Carotenoids have protective effect. Microorganisms excreting pigment to medium are as sensitive to U.V. as colorless organisms.		Imshenetskii	1946
Low temp. alone does not destroy bacteria but appears to favor longevity.		Keith	1913
Storage temperature approaching 0 C. results in a greater decrease in bacterial numbers than that observed at lower temperature.		Kiser	1942
120 C. steam is effective in sterilizing spores of soil bacteria		Konrich	1935
28 C., sea water	4 d.	Lipman	1926
Organisms survive equally well at temp. colder than solid CO_2. Survive temp. ranging from that of liquid oxygen (—183 C.) to that of liquid He (—296 C.) In rapid freezing the water in the cell is not changed to ice crystals but to a glass-like amorphous mass and this results in less injury than freezing at slower rates.		Luyet	1938
Most organisms live longer under sterile paraffin oil.		Morton	1938
23 cultures enteric bacteria from suspected food preserved on nutrient agar. Original character unchanged 11–37 yrs.		Pergola	1950
Death during storage is a function of storage temp. Moisture presence most important factor contributing to loss of viability. Presence of O_2 deleterious. Dry nitrogen atmosphere superior. Survived best when protective colloids such as serum and broth used. Survival lowest in saline. Young cultures more resistant to drying.		Proom	1949
Viable in frozen fruits after 3 yrs. at 15 F. Agar slants held at 16 F. for 1 yr.		Smart	1935
7–10 C. below optimal temp.	Maximum tolerance observed	Van Eseltine	1949
High temp. resulted in spores of increasing resistance in peptone water with added salts of magnesium phosphate.		Williams	1929

TABLE C11—(Continued)

The Survival of Microorganisms in Culture (General)

Factor(s)	Survival	Reference	
GENERAL, Continued			
Sodium favors viability but toxic in high conc. Calcium opposite effect of sodium in low conc. Pb and Hg, though toxic, stimulate viability in high dilution.		Winslow	1928
Marine bacteria very sensitive to heat		Zobell	1939
Cult., —15 C.	160 wks.	Tanner	1928

TABLE C12

The Survival of Mycobacterium Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
M. tuberculosis			
Frozen culture, desiccated, refrigerator	3 yrs.	Cohn	1939
Natural cultures	6 mos.–1 yr.	Cohn	1939
Incubator, desiccated	6 mos.–1 yr.	Cohn	1939
Drying	Shortens viability	Corper	1923
17 years desiccation in vacuo on Lowensteins or Dubos medium	9 strains, none grew	Frobisher	1949
Petroffs medium, in evac. bottle in ice box 11 mos.	10 strains, 11 mos.	Harris	1933
Desiccated in vacuo	17 yrs.	Frobisher	1949
Dried	4–5 yrs.	Proom	1949
LIQUID			
M. tuberculosis			
NaCl soln., 0.9%, inject into guinea pig	24–31 d.	Bartel	1908
NaCl soln., 0.9%, 2 h. after G.P. inoc.	43 d.	Bartel	1908
NaCl soln., 0.9%, 8 h. after inoc.	29 d.	Bartel	1908
NaCl soln., 0.9%, 24 h. after inoc.	100 d.	Bartel	1908
Nacl soln., 0.9%, 48 h. after inoc.	88 d.	Bartel	1908
Old culture	43 d.	Bartel	1908
Dist. water and glycerine inject into G.P.	13 d.	Bartel	1908
Dist. water and glycerine inject after 2 d.	.....	Bartel	1908
Dist. water and glycerine inject after 33 d.	155 d.	Bartel	1908
Potato with glycerinated bouillon susp. in Lowensteins med. 1 C.	567 d.	Boquet	1943
Petroffs gentian violet and egg	Viable 4–8 mos.	Corper	1923

TABLE C12—(Continued)

The Survival of Mycobacterium Species in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Buffered isotonic coln. pH 7			
Temp. 60 C.	Few min.	Corper	1923
Temp. 50 C.	5 h.	Corper	1923
Temp. 45 C.	8 d.	Corper	1923
Broth, whole egg, 50 C.	5 h.	Corper	1938
Broth, whole egg, 55 C.	1 h.	Corper	1938
Broth, whole egg, 60 C.	15 min.	Corper	1938
Petroffs gentian violet or 5% glycerol agar in ice box or incubator	4–8 mos.	Corper	1923
Physiol. saline, 5–10 C.	Alive and virulent >1 yr.	Feldman	1932
Saline, susp. —76 C. frozen, dried	>6 mos.	Glover	1946
Dorsets	6 yrs.	Gloyne	1920
Purified casein and glycerol, 37 C.	2 yrs.	Hastings	1923
.85% NaCl, —252 C.	50 h.	Kadisch	1931
M. tuberculosis			
Bouillon, R.T., Petri dish	5 d.	Kirstein	1902
Liquid air in steel tube, —30 C. stored in dark ice box (Avian)	No growth after 6 yrs.	Kyes	1939
—192 C., liquid air (Avian)	Did not destroy all viable bacilli but decreased rate of surviving bacilli multiplicat. in culture and produced TB in animals	Kyes	1939
Stored daily for 21 hrs. in CO_2 bath	All animals showed + in spleen following infection	Kyes	1939
Culture stored 6 yrs.	Inj. into rabbits (4 cc.) all died 3–4 wks.	Kyes	1939
—3 C. in PO_4 soln.	Failed to show any effect on 6 wk. old chicks. Lost pathogen. after 1 h. R.T.	Kyes	1939
200 X alternate freezing in liquid N_2 (—195 C.) and thawing in water at 34–36 C.	Still showed some viable organisms	Kyes	1939
Egg medium	Growth	Larson	1922
1% castor oil, soap soln.	No growth	Larson	1922
0.8% NaCl soln.	3 d.	Moriya	1909
Beef decoction	9 mos.	Moriya	1909
Glycerol bouillon	9 mos.	Moriya	1909
.8% NaCl 37 C.	98 d.	Moriya	1909
.8% NaCl ice box	152 d.	Moriya	1909
Beef broth infusion, ice box	156 d.	Moriya	1909
Glycerin bouillon, ice box	156 d.	Moriya	1909
Glycerine agar, 37 C., sealed and incub. (Avian)	Viable 20 d. Non-viable 30 d.	Potter	1939
Glycerine agar, 37 C. culture, 2–4 wks. sealed in glass (human)	Infectious power retain	Potter	1939

TABLE C12—(Continued)

The Survival of Mycobacterium Species in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Glycerine agar cult. 38 C. glass sealed, incub. (bovine)	Loss of infect., 2 mos.	Potter	1939
Hohn, Lowenstein, Petragnana deprived of O_2, stored in dark, 2–5 wks. (human)	Non-viable 1 mo.	Potter	1942
.85% NaCl	800 d. does not infect guinea pig	Potter Pugnani	1942 1929
Glycerine agar tubes with ozonized air, 37 C.	No change in 10 d.	Ransome	1901
M. tuberculosis			
Physiol. saline soln. .85%, near freezing	310–330 d.	Shope	1926
Lymph node emulsion in salt soln.	87 d.	Webb	1921
Egg	30–50 min.	Weinzirl	1907
SOLID			
M. tuberculosis			
Glycerol agar, 37.5 C.	4–8 mos. (max. 16 mos.)	Cooper	1923
Glycerol agar	Dead in 6 yrs. (M. phlei dead in 4 yrs.)	Gloyne	1920
Petroffs medium in bottle evac., left in ice box 11 mos.	(Human) 11 mos. (Bovine) 11 mos. (Avian) 11 mos.	Harris Harris Harris	1933 1933 1933
Agar, pH 6.6	Grew well Heating decreases number of survivors able to grow during lag phase. Unless grown at pH 7.6 more survivors grew but the lag period was not decreased	Isaacs	1930
Artificial media	>10 yrs.	Karwacki	1928
Solid media in pyrex tube sealed in vacuo	(Avian) viable 8 mos.	Potter	1935
Pyrex tube, R.T., sealed in vacuo	(Str. P4) viable 80 d.	Potter	1935
Dark, 37 C.	5 mos.	Potter	1935
GENERAL			
M. tuberculosis			
40–50 F.	7–19 mos. Bovine more resistant than human	Cooper	1923
37 C. incub. in glycerol broth	12 yrs., 42% human viab. 12 yrs., 44% bovine viab.	Cooper	1923
—9.4 to —19.9 C.	Viable 1st 24 h.	Homma	1927
—1.0 to —8.0 C.	Viable 2nd 24 h.	Homma	1927
—7.0 to —14.3 C.	Viable 3rd 24 h.	Homma	1927
—17.3 to —29.2 C.	Viable 4th 24 h.	Homma	1927
—10.6 to —23.9 C.	Viable 5th 24 h.	Homma	1927
—13.4 to —26 C.	Viable 1st 24 h.	Homma	1927
—11.5 to —24.3 C.	Viable 2nd 24 h.	Homma	1927

TABLE C12—(Continued)

The Survival of Mycobacterium Species in Culture

Factor(s)	Survival	Reference	
GENERAL, Continued			
—8.7 to —17.4 C.	Viable 3rd 24 h.	Homma	1927
Below 0 C.	623.5 h.	Homma	1927
Above 0 C.	216.5 h.	Homma	1927
Mixed with quinine	Requires 25 min. exp. to U.V.	Mayer	1924
Deprived of O_2 and H_2O for 24 hrs.	Survive as viable pathogens	Potter	1935
Dark, no O_2 R.T., desic.	(Str. P4) 2 yrs.	Potter	1937
Dark, 37 C., H_2 desic.	(Str. P7) 1 yr.	Potter	1937
M. tuberculosis			
Deprival of O_2 kills tubercle bacilli. Rapid desiccation favors survival. Avian bacilli at body temp. survive for more than 1 yr. with only trace of O_2 and H_2O while at R.T. they survive 2 yrs.		Potter	1937
pH 2.55	168 h.	Prudhomme	1935
pH 1.95	48 h.	Prudhomme	1935
pH 0.97	24 h.	Prudhomme	1935
Nucleic acids have no specific action on the tb bacillus. Tb bacillus destroyed by pH <2. Acetic acid has no effect.		Prudhomme	1935
100 C.	.....	Ruska	1941
Ice box, near freezing	330 d.	Shope	1926
Cult., protected from light	3 wks.	Simmons	1923
Darkness and moisture conducive to prolongation of life, while dryness and thermostat temp. hastened destruction.		Twitchell	1906
2 C.	12 mos.	Vidal	1934
BCG, DRIED, LYOPHILIZED			
5% gelatin, 4 C.	12 mos.	Ungar	1949
5% gelatin, 26 C.	12 mos.	Ungar	1949
Sterile serum, 4 C.	12 mos.	Ungar	1949
Sterile serum, 6 C.	12 mos.	Ungar	1949
Lowensteins egg med., 38 C., before freeze drying	No difference in number of colonies between, before and after	Van Deinse	1950
Keeps viability in 50% glucose better than lactose, amylase or serum in cylinder with paraffin oil, —50 C.		Van Deinse	1950
R.T.	Diminished number living organisms, 20% at end of 1st mo.	Van Deinse	1950
Stacked in ice box, 5 C.	No mortality during 1st 3 mos., 10% after 6 mos.	Van Deinse	1950
Stored at R.T.	2 mos.	Van Deinse	1950
BCG, LIQUID			
Lowensteins egg medium, 1:10 dil., 2-4 C.	2 yrs.	Birkhaug	1951

TABLE C13

The Survival of Neisseria Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
Neisseria intracellularis			
Dried on garnets and glass in dark	24 h.	Flugge	1905
Diffuse daylight	10 h.	Flugge	1905
Dark	25 h.	Flugge	1905
Dried	4–5 yrs.	Proom	1949
N. gonorrhea			
Dried	4–5 yrs.	Proom	1949
N. species			
Dried	4–5 yrs.	Proom	1949
Drying	Little resistance	Rhodes	1950
DRIED, LYOPHILIZED			
N. intracellularis			
Dried in frozen state	18 yrs.	Elser	1935
Frozen and dried 16 hr. cult. on 10% rabbit blood pneumococcus agar plate in vacuo	151 d.	Rake	1935
Above with 2 wk.-old stock strains	>89 d.	Rake	1935
Above with 4 fresh strains	>151, >141, >89, >41 d.	Rake	1935
2 cc. broth	>2 mos.	Swift	1921
N. gonorrhea			
Dried in frozen state	18 yrs.	Elser	1935
LIQUID			
N. intracellularis			
Bouillon, diffuse light, dried, ascitic agar, 37 C.	24 h. (Raul str.)	Beltencourt	1904
Bouillon, 20–25 C., 80% RH	3 h.	Beltencourt	1904
Bouillon susp. of 24 h.			
with ascitic agar, 50 C.	3 min.	Beltencourt	1904
with ascitic agar, 55 C.	1 min.	Beltencourt	1904
with ascitic agar, 60, 70, 80 C.	30 sec.	Beltencourt	1904
with ascitic agar, 100 C.	30 sec.	Beltencourt	1904
Bouillon culture, 0–70 C.	1 mo. or longer	Beltencourt	1904
Ringers, 20 C.	8–10 d.	Beltencourt	1904
Saline, 50 C.	Poor	Flexner	1907
Saline, 20 C.	Fair	Flexner	1907
Saline, 37 C.	Good	Flexner	1907
Physiol. saline, R.T., abs. drying in vacuo	Viable for months	Otten	1930
Pure, undil. neutral glycerine, —15 C.	No change in viability 2 yrs.	Pabst	1935
Saline broth, dried at R.T., or from frozen state	42%	Proom	1949
Broth, R.T.	.69%	Proom	1949
Saline, —78 C.	36.2%	Proom	1949
Sterile serum sealed with paraffin layer	16 mos.	Ungerman	1918
N. intracellularis			
Culture, 104th generation	3–4 yrs.	Von Albrecht	1901

TABLE C13—(Continued)

The Survival of Neisseria Species in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
N. gonorrheae			
Pigeon blood, pH 6.6	No growth, 12 d.	Lal	1925
Pigeon blood, pH 6.8	108 d.	Lal	1925
Pigeon blood, pH 7.0	108 d.	Lal	1925
Pigeon blood, pH 7.2	123 d.	Lal	1925
Pigeon blood, pH 7.4	159 d.	Lal	1925
Pigeon blood, pH 7.6	189 d.	Lal	1925
Pigeon blood, pH 7.8	189 d.	Lal	1925
Pigeon blood, pH 8.0	76 d.	Lal	1925
Lactic acid plates in ice box 4–7 C., preincub. in moist chamber	Viable 9–15 d.	Lorentz	1924
Physiol. saline, R.T., abs. drying in vacuo	Viable for mos.	Otten	1930
Gentian violet, 20–26 C.	24 h.	Peizer	1949
Physio. NaCl, R.T., on ascitic agar, 24 h. cult.	Recov. 79.2%, 6 h.	Pieper	1930
Ringers soln., R.T., on ascitic agar, 24 h. cult., 18 C.	Recov. 71.8% in 6 h.	Pieper	1930
Ringers soln., R.T., on ascitic agar, 24 h. cult., 22 C.	Recov. 30.7% in 24 h.	Pieper	1930
Physiol. NaCl, 48 h. cult., on ascitic Levinthal med., 18 C.	Recov. 57.9% in 6 h.	Pieper	1930
Physiol. NaCl, 48 h. cult., on ascitic Levinthal med., 22 C.	Recov. 21% in 24 h.	Pieper	1930
Ringers soln., 48 h. cult., on ascitic Levinthal med., 18 C.	Recov. 70.9%, 6 h.	Pieper	1930
Ringers soln., 48 h. cult., on ascitic Levinthal med., 22 C.	Recov. 16%, 24 h.	Pieper	1930
Saline broth, dried at R.T.	1.5%	Proom	1949
Saline broth, dried from frozen state	15%	Proom	1949
Broth, R.T., saline, —78 C.	0.93%	Proom	1949
Sterile serum sealed with paraffin layer	7–8 wks.	Proom	1949
SOLID			
N. intracellularis			
Serum agar, 37 C.	8 wks.	Dharmendra	1940
Pigeon blood agar, 37 C.	10 wks.	Dharmendra	1940
Serum agar, 3.5–8.5 C.	27 wks.	Dharmendra	1940
Pigeon blood agar, 3.5–8.5 C.	31 wks.	Dharmendra	1940
Yeast agar, 37 C.	>5 mos.	Eberson	1920
Agar surface, 2 C.	Poor	Flexner	1907
Agar surface, 32–37 C.	Good	Flexner	1907
N. intracellularis			
Glucose agar slant, —15 C.	No loss of viab. 8 mos.	Pabst	1935
Gelatin 37 C., 1% acid added to phenolphthalein	>7 mos.	Worth	1919
N. gonorrheae			
Gelatin 37 C., 1% acid added to phenolphthalein	>8 mos.	Worth	1919

TABLE C13—(Continued)

The Survival of Neisseria Species in Culture

Factor(s)	Survival	Reference	
GENERAL			
N. intracellularis			
Various nutritive substances alter the survival period		Foa	1887
37 C.	>86 d.	Lal	1921
Survival shortened at 37 C. and prolonged in ice box, 6–10 C.		Miller	1944
.....	4 d.	Murray	1929
—5 C.	5 d.	Pabst	1935
Icebox, 0–5 C.	48 h.	Von Albrecht	1901
Icebox, 65 C.	½ h.	Von Albrecht	1901
Icebox, 80 C.	Few min.	Von Albrecht	1901
Icebox, 100 C.	Immediate	Von Albrecht	1901
Stoppered with gutta percha, 19–21 C.	4–5 d.	Von Albrecht	1901
Dried and stoppered with gutta percha, incub. temp.	185 d.	Von Albrecht	1901
N. gonorrheae			
40 C.	99.7% 10 hrs.	Carpenter	1933
41 C.	99% 4–5 hrs.	Carpenter	1933
41 C.	100% 11–23 hrs.	Carpenter	1933
41.5 C.	7–20 hrs.	Carpenter	1933
42 C.	5–15 hrs.	Carpenter	1933
50 C.	Few min.	Carpenter	1933
Gentian violet, ice box	Stored 8 wks.	Peizer	1949
—20 C.	10 d.	Luniere	1914
—195 C.	24 h.	Luniere	1914

TABLE C14

The Survival of Pasteurella Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
Pasteurella pestis			
37 C.	Most dead in 3 d.	Gladin	1898
Bubo juice, R.T., exposed to sun, dried	3–4 h.	Kitasato	1894
Bubo juice, R.T., dried on coverslip	<4 d.	Kitasato	1894
Dried	Viable 2 h.	Tinker	1930
P. tularensis			
Susp. in mixt. of beef infusion, cystine and rabbit blood, dried, sealed	4 yrs.	Miller	1946
P. species			
Dried	4–5 yrs.	Proom	1949
LIQUID			
P. multocida			
Broth culture, R.T., in sealed ampule	(Avicida) 2 yrs.	Nobrega	1950

TABLE C14—(Continued)

The Survival of Pasteurella Species in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Broth culture, 2–4 C. in sealed ampule	(Avicida) 1 yr.	Nobrega	1950
Broth culture, 37 C.	(Avicida) 1 yr.	Nobrega	1950
Serum, 37 C.	(Avicida) 4.5 h.	Panisset	1925
P. pestis			
Pus on cover slip, 28–30 C., innoc. to bouillon	4 d.	Abel	1897
Pus and culture on cover glass, R.T.	Still viable 6–9 d.	Abel	1897
Pure undil. glycerine, —150 C.	Fully virulent 2 yrs. and 14 mos. Slightly, 2 yr. and 7 mos. Dead 3 yrs.	Francis	1932
Physiol. serum	5 hrs.	Jacotot	1926
Bouillon cult., —31 C.	5½ mos.	Kasansky	1899
Bouillon cult., —24 C.	Still viable for 9, 32, and 35 d. respectively for 1st and 2nd subcult. 33d for 3rd subcult.	Kasansky	1899
Bouillon cult., —1.8 C.	6th to 15th subculture, 12 mos.	Kasansky	1899
Bouillon cult., 1 yr. and 9 mos. old	Recov. 58.6%, 6½ yrs.	Lenskaja	1931
Bouillon, 48 and 24 hr. cult.	7 d.	Lenskaja	1931
Sealed cult. tube of Hammorek medium, 100 C. dark	Living >4 yrs.	Schultz	1901
Bouillon loops, 50 C.	Innoc. with original cult. which is 2 yrs and 10 mos. old. 1 to several d.	Schultz	1901
Twiks soln.	2 h.	Tinker	1930
SOLID			
P. pestis			
Gelatine, 15 C.	3–4 d.	Abel	1897
Gelatine, 8–10 C.	6 d.	Abel	1897
Agar and gelatine cult.	.....	Abel	1897
Plain agar 10 C., sealed for 9 yrs.	Viable at end of 9 yrs. with full virulence	Francis	1932
Plain agar 10 C. Subcultured every 3 mos.	Viable but non-virulent at end of 9 yrs.	Francis	1932
Culture 5–10 C., innoc. into cork stoppered bottle	25 yrs.	Francis	1949
Beef infusion agar, 10 C. on surface without transfer	Virulent 20 yrs.	Francis	1943
Agar cult. in sun, 39 C.	2¾ hrs.	Gladin	1898
Agar plate in sun, 40–46 C.	5½ hrs.	Gladin	1898
Agar cult., —1.8 C.	1st subcult. still viable 4 mos.	Kasansky	1899
Agar cult., —31 C.	2nd and 3rd subcult. 5–5½ hrs.	Kasansky	1899

TABLE C14—(Continued)

The Survival of Pasteurella Species in Culture

Factor(s)	Survival	Reference	
GENERAL			
P. pestis			
Artificial cold, —22 C.	2 h.	Gabritschewsky	1899
Natural cold, 0 to —20 C.	12–40 d.	Gabritschewsky	1899
Freeze and thaw daily, —20 C.	Still alive 40 d.	Gladin	1898
Stored 37 C.	1% survived 4 yrs.	Stamp	1947
Ice box culture	10 yrs., 5 mos.	Wilson	1913
P. pleurisepticus			
Physiol. NaCl, R.T., abs. dried over H_2SO_4 in vacuo	Viable for months	Otten	1930
P. pseudotuberculosis			
Sealed and viable	Viable for a number of yrs.	Merlini	1938
P. species			
Physiol. NaCl, R.T., abs. dried over H_2SO_4 in vacuo	Innoc. into blood or serum, viable mos.	Otten	1930
P. tularensis			
Eggs, freezing	Recov. 1%, 3 mos.	Downs	1947
Factors leading to population changes. 1) Type and comp. of med., 2) initial pH, 3) Innoculum size, 4) One or more factors present in old cult. filtrates		Eigelbach	1951

TABLE C15

The Survival of Protozoa and Metazoa in Culture

Factor(s)	Survival	Reference	
LIQUID			
E. histolytica			
Ringers' fluid and solid rice starch, body temp.	3 wks.	Dobell	1926
Dist. H_2O, tap water, Ringers, physiol. saline, R.T.	14 d.	Dobell	1926
10–20 C., R.T.	3 d.	Dobell	1926
Sterile dist. water, R.T.	26 d.	Dobell	1927
N/20 HCl, R.T.	<30 min.	Dobell	1927
N/40 HCl, R.T.	At least 1 h.	Dobell	1927
N/15 HCl, R.T.	As long as 3 h.	Dobell	1927
N/20 HCl, 37 C.	1 h.	Dobell	1927
Plasmodium gallinaceum			
Susp. saline extract, R.T. in washed chicken rbc.	Little loss of infectivity 72 hrs.	Whitman	1948
Trypanosoma cruzi			
N.N. medium, R.T.	>6 yrs.	Packchanian	1943
Trichomona vaginalis			
R.T.	8 d.	Mohr	1937

TABLE C15—(Continued)

The Survival of Protozoa and Metazoa in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Bact. free in serum, Ringers soln. over liver infusion agar slants, 37 C.	4–6 d.	Trussel	1947
Cysteine, peptone broth, liver, maltose, R.T.	13 d.	Trussel	1947
SOLID			
S. mansoni			
Horse serum	14–18 d.	Ross	1950
Serum ultra filtrate	10–12 d.	Ross	1950
100% N_2	5 d.	Ross	1950
L. donovani			
Blood agar, R.T.	4 mos.	Packchanian	1943
T. americanum			
Blood agar, R.T.	4 mos.	Packchanian	1943
T. avium			
Blood agar, R.T.	3 mos.	Packchanian	1943
T. duttoni			
Blood agar, R.T.	4 mos.	Packchanian	1943
T. lewisii			
Blood agar, R.T.	4 mos.	Packchanian	1943
T. malophagium			
Blood agar, R.T.	4 mos.	Packchanian	1943
T. rotatorium			
Blood agar, R.T.	4 mos.	Packchanian	1943
T. vaginalis			
Semi-dry state	6 hrs.	Vazques-Calet	1936
GENERAL			
E. histolytica			
Powdered starch, R.T.	10 d.	Walker	1913
T. vaginalis			
3–5 C.	21 d.	Fischer	1935
10–15 C.	2–3 wks.	Florent	1948
Lactic acid .27 to .102 mg%, ice box	3 d.	Furushima	1936
Trypanosoma equiperdium			
—20 C.	3¼ hrs.	De Jong	1922
—145 C.	45 min.	De Jong	1922
—191 C.	31 d.	De Jong	1922
Trypanosoma evansi			
—20 C.	1¼ hrs.	De Jong	1922
—191 C.	9 min.	De Jong	1922
Ascaris lumbricoides			
—6 to —17 F.	Inactive 20 d.	Cram	1924
12–18 F.	Active 30 d.	Cram	1924
10–18 F.	Few active 6 d.	Cram	1924
—2 to —16 F.	Few active 6 d.	Cram	1924
—6 to —17 F.	40 d.	Cram	1924
60 C.	5 min.	Ohba	1923
70 C.	10 sec.	Ohba	1923

TABLE C16

The Survival of Rickettsiae in Culture

Factor(s)	Survival	Reference	
DRYING, LYOPHILIZED			
R. tsutsugamushi			
Soln. 0.2 M. sucrose plus buffer salts	Superior to skim milk medium	Jackson	1951
LIQUID			
Coxiella burneti (Dyer nine mile and Henzerling str.)			
Saline susp. yolk sac. 60 C.	30 min.	Ransom	1951
Saline susp. yolk sac. 50 C.	15 min.	Ransom	1951
R. mooseri, and prowazeki			
Yolk sac material, —72 C., .005 glutamate, pH close to 7, basal salt soln., high in K ions, .1 to 1% serum albumin	Survival favored by these	Bovarnick	1950
Tissue cult., in serum with Tyrodes, 37 and —20 C.	Several mos.	Nigg	1935
Tissue cult., in serum with Tyrodes, 20 and —40 C.	Week or two	Nigg	1935
R. prowazeki			
Sterile skim milk, 26–28 C.	24 h.	Anderson	1944
Broth, 26–28 C.	6 h. (Breine str.)	Anderson	1944
20% normal yolk sac, 26–28 C.	50% mortality, 6 hrs. (Bogota epidemic str.)	Anderson	1944
Tyrodes soln. 26–28 C.	6 h.	Anderson	1944
Tyrodes, G.P. serum, 26–28 C.	6 h.	Anderson	1944
56 C.	30 min.	Rivers	1948
Blood spec. 2–4 C.	1 or more h.	Rivers	1948
Blood spec. 37 C.	Few hrs.	Rivers	1948
Desiccated, R.T.	Few hrs.	Rivers	1948
G.P. blood, R.T.	Few hrs.	Rivers	1948
Carbon tet. or benzene, CS_2, 25% NH_3, ether, cresol, ethylene Cl_2, tetramine	Viable >1 hr. <7 min.	Schlote	1948
Physiol. saline with tap water	Affects viability deleteriously	Topping	1940
R. species			
Aqueous susp.	40 hrs.	Latarjet	1951
Chick embryo dil. 1/10 N. saline, 50 C.	1 wk.	Payzin	1947
Chick embryo dil. with Tyrodes and horse serum	Not viable after 20 d.	Payzin	1947
Chick memb., —14 C., with carbon snow in presence of P_2O_5, sealed under H_2	13 d.	Payzin	1947
R. rickettsi			
Pure glycerol, —10 C.	10 mos.	Spencer	1924
Rocky Mtn. Spotted Fever			
Desiccated, R.T.	Few hrs.	Rivers	1948
Guinea pig blood, R.T.	1 wk.	Rivers	1948
Rocky Mtn. Spotted Fever			
G.P. blood, cold room	2 wks.	Rivers	1948
G.P. blood, brain and spleen, —7 C.	1 yr.	Rivers	1948

TABLE C16—(Continued)

The Survival of Rickettsiae in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Typhus			
Broth, pH 7.69	Trace 48 hrs.	Elford	1944
Fermented broth, pH 8.10	Trace 96 hrs.	Elford	1944
Saline, pH 6.85	Trace 48 hrs.	Elford	1944
Serum and saline, pH 7.93	Trace 48 hrs.	Elford	1944
Water, pH 6.95	Trace 2 hrs.	Elford	1944
Peptone water, pH 7.06	Trace 2 hrs.	Elford	1944
Tyrodes soln., pH 8.18	Trace 2 hrs.	Elford	1944
GENERAL			
R. burneti			
—70 C.	Long time	Rivers	1948
Infection spread by means of air borne droplets		Commen. on Resp. Dis.	1946
R. prowazeki			
Dry heat, 39 C. —41 C.	1 h.	Schlote	1948
Scrub typhus			
—70 C.	Long time	Rivers	1948
Typhus			
77 C., pH 5.8–8.8	4 mos.	Elford	1944
—10 or 0 C.	2–3 days	Elford	1944
37 C.	Trace 48 hrs.	Elford	1944
23 C.	Trace 2 hrs.	Elford	1944
0 C.	Trace 48 hrs.	Elford	1944
—10 C.	Present 8 d.	Elford	1944
—77 C.	Present 8 d.	Elford	1944

TABLE C17

The Survival of Salmonella Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
S. paratyphi			
Dried	Viable >2 h.	Orskov	1925
Nutrient gel with ascorbic acid. Desic. 3 d.	Recov. 55.7%, 23 d.	Stamp	1947
Nutrient gelatin and ascorbic acid. Dried over P_2O_5 at 100–300 mm. Hg.			
Stored in vacuo, R.T.	>4 yr. survival	Stamp	1947
S. Species			
Dried	4–5 yrs.	Stamp	1947
DRIED, LYOPHILIZED			
S. choleraesuis			
Broth culture	10 yrs.	Schoening	1949
LIQUID			
S. choleraesieus			
Glucose-peptone water	7 d.	Oshii	1920

TABLE C17—(Continued)
The Survival of Salmonella Species in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
S. enteriditis			
NaCl soln.	4½ wks.	Karaffa-Korbutt	1912
S. hiss	1,283 d.	Lal	1925
Glucose peptone water	5–7 d.	Oshii	1920
NaCl soln.	Recov. 0, 4½ wks.	Stadler	1899
S. morbificans			
NaCl soln.	3 wks.	Karaffa-Korbutt	1912
NaCl soln.	Recov. 0, 3 wks.	Stadler	1899
S. paratyphi A			
Tryptic digest	3,259 d.	Lal	1925
Bouillon, 63 C.	Viable 1–4 min.	Orskov	1925
Saline, 0.9%, 62 C.	<5 min.	Orskov	1925
Lactose peptone water	3–5 d.	Oshii	1920
NaCl soln., R.T., dried in vacuo	Viable mos.	Otten	1930
S. paratyphi B			
Muttan, agar	3,259 d.	Lal	1925
Bouillon 63 C.	4 min.	Orskov	1925
Lactose peptone water	3–6 d.	Oshii	1920
NaCl soln., R.T., dried in vacuo	Viable mos.	Otten	1930
S. schottmulleri			
Broth and water, frozen	17 d.	Thomas	1925
S. species			
Bouillon cult., in glass capillaries	>1 yr.	Wesselinoff	1949
S. typhimurium			
Bouillon and beer wort, exposed to U.V. 10–15 min.	1–15 sec.	Gilles	1935
SOLID			
S. enteriditis			
Transmitted by rat fleas		Eskey	1949
S. paratyphi B			
Blood gelatin	>649 d.	Lal	1921
S. Paratyphi B			
Crust of rye bread, R.T.	.....	Bachmann	1943
S. typhimurium			
Gelatin	98 d.	Glass	1946
GENERAL			
S. choleraesuis			
.....	>657 d.	Lal	1921
.....	1,562 d.	Lal	1925
S. gallinarum			
Stored at 37 C.	28.4% surv. 4 yrs.	Stamp	1947
S. paratyphi C			
.....	50 d.	Kister	1928
Stored at 37 C.	14% surv. 4 yrs.	Stamp	1947
S. typhimurium			
Stored at 37 C.	18.4% surv. 4 yrs.	Stamp	1947
S. typhisuis			
Stored at 37 C.	16.2% surv. 4 yrs.	Stamp	1947

TABLE C18—(Continued)

The Survival of Salmonella typhosa in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
Dried in thin layers	5–15 d.	Osler	1901
Dried in thick layers	Mos.	Osler	1901
Vacuum dried, —195 C.	4 d.	Shattock	1912
Air dried, dark	4 d.	Shattock	1912
DRIED, LYOPHILIZED			
Standardized susp., —192 C.	10 d.	Turner	.....
LIQUID			
.85% NaCl, freezing	>6 d.	Bolten	1918
Bouillon culture, continuous freezing @ —20 to —16 C.	Survived 4½ mos.	Brehme	1901
Watery susp. of fresh cult. placed on Petri dish in sunlight	Innoc. 100. 95–99% reduct. in 10–15 min. 100% reduct. ½–4 hrs.	Clark	1903
1% soap soln., 4–8 C.	12 h.	Jolles	1895
6% soap soln., 4–8 C.	15 min.	Jolles	1895
1% soap soln., 18 C.	24 h.	Jolles	1895
6% soap soln., 18 C.	30 min.	Jolles	1895
5% soap soln., 18 C.	1 h.	Jolles	1895
3% soap soln., 18 C.	12 h.	Jolles	1895
Bouillon susp., R.T., fall on Petri dishes	24 h.	Kirstein	1902
Tryptic digest	3,838 d.	Lal	1925
—185 C., liquid air	No impaired vitality 20 h.	MacFayden	1899
Broth emulsion with liquid air	No impaired vitality 7 d.	MacFayden	1900
Sealed tubes, liquid air	6 mos.	MacFayden	1900
Liquid H_2, —252 C.	10 h.	MacFayden	1900
Beef-peptone agar in sun	10 min. to 1 h.	Minck	1896
Bouillon, 63 C.	Viable 1–2 min.	Orskov	1925
Lactose peptone water	3–5 d.	Oshii	1920
Thick susp., R.T., dried in vacuo over H_2SO_4	Innoc. to blood or serum agar, 2–5% survived 24–48 h.	Otten	1930
NaCl soln., R.T., dried in vacuo	Innoc. to blood or serum agar, viable mos.	Otten	1930
Bouillon, neutral pH	82 d.	Remy	1900
Peptonized beef bouillon freezing, —17.8 C.	Recov. 1/45, 2 h.	Smith	1905
Peptone beef bouillon freezing, —190 C. in liquid air	Recov. 2/60, 2 h.	Smith	1905
Peptonized beef bouillon, frozen —17.8 C.	99.5% killed 2 h. .5% recov. 2 h.	Smith Smith	1905 1905
Huntoon broth, frozen	13 d.	Thomas	1925
Susp. in agglut. immune serum	No effect on surv. time.	Tinti	1923
Emulsion 24 hr. bouillon cult. in salt soln. heated at 80 c. for 10 min.	2–8 hrs. (6X as much needed to kill spores)	Weinzirl	1914
Liquid air in glass	Recov. 25%, 2 h.	White	1901

TABLE C18

The Survival of Salmonella typhosa in Culture

Factor(s)	Survival	Reference	
SOLID			
Crust of rye bread, R.T.	4½ mos.	Buchanan	1943
Agar, full radiation Hg. arc. under glass, water cooled	10 min. at 10 cm.	Bazzoni	1914
Agar and gelatin plates	1½ h. (July, Mar., Aug.)	Dieudonne	1894
Agar and gelatin plates	2½ h. (Nov.)	Dieudonne	1894
Agar and gelatin plates	4½ h. (Winter)	Dieudonne	1894
Salt media	91 d.	Frank	1941
Nutrient gel tubes, 22 C.	No change in 8 d.	Ransome	1901
Action of H ions on microorganisms is very rapid and is increased by raise in temp. By changing the temp. from 20 to 45 C. the pH at which typhoid bacilli are killed shifts from 4.2 to 5.1.		Sierakowski	1924
Gelatin, 20 C., 1% acid added to phenolphthalein	>8 mos.	Worth	1919
GENERAL			
Artificial media	>5 wks.	Houston	1912
.....	>929 d.	Lal	1921
113 C.	Living	Lal	1923
120 C.	Dead	Lal	1923
R.T., dark	2,906 d.	Lal	1923
60 C.	10 min.	Osler	1901
—50 C.	18 wks.	Osler	1901
—2 to —7 C.	22 wks.	Park	1924
45 C.	Dried after 46th change	Spencer	1942
Storage at R.T.	50% surv., 4 yrs.	Stamp	1947
Storage at R.T.	5.6% surv., 4 yrs.	Stamp	1947
Storage at R.T.	18.8% surv., 4 yrs.	Stamp	1947
Storage at R.T.	1.3% surv., 4 yrs.	Stamp	1947
Storage at R.T.	16.7% surv., 4 yrs.	Stamp	1947
Sunlight has no appreciable inhibiting effect		Stimson	.....
Ice cream, —19 C.	1 yr.	Tanner	1928
Excess lime, pH 9–9.5	>540 min.	Wattie	1943
Excess lime, pH 9.5–10	>540 min.	Wattie	1943
Excess lime, pH 10–10.5	>540 min.	Wattie	1943
Excess lime, pH 10.5–11	240 min.	Wattie	1943
Excess lime, pH 11–11.5	120 min.	Wattie	1943

TABLE C19

The Survival of Serratia Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
S. spp.			
Dried	4–5 yrs.	Proom	1949
DRIED, LYOPHILIZED			
S. marcescens			
Stored at R.T., dark	Survivals 100%. Viability lost when stored in air	Naylor	1946

TABLE C19—(Continued)
The Survival of Serratia Species in Culture

Factor(s)	Survival	Reference	
DRIED, LYOPHILIZED, Continued			
S. rubidaeum			
Frozen at —24 C.	82.2% killed in 1st freezing	Stille	1943
LIQUID			
S. marcescens			
Standard nutrient broth, R.T.	29–21 yrs.	Deacon	1932
Bouillon susp., R.T.	24 h.	Kirstein	1902
Broth, —13 to —15 C.	Recov. .1%, 8–9 wks.	Tanner	1928
Salt soln., —13 to —15 C.	Recov. .1%, 12–16 wks.	Tanner	1928
Broth, before centrifug.	100% survival	Winslow	1927
Broth, after centrifug.	44% survival	Winslow	1927
Broth, 1 hr. later	37% survival	Winslow	1927
SOLID			
S. marcescens			
Agar and gelatin plates, March, July, Aug.	1½ h.	Dieudonne	1894
GENERAL			
—10 to 1 C. (S. marcescens)	51 d.	Hilliard	1918
S. marcescens			
.....	658 d.	Lal	1921
Stored at 37 C.	16% recov., 4 yrs.	Stamp	1947

TABLE C20
The Survival of Shigella Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
S. spp.			
Dried	4–5 yrs.	Proom	1949
Desiccated	20–25 d.	Vaillard	1903
LIQUID			
S. dysenteriae			
Bouillon	920 d.	Lal	1921
Mutton, agar slope	1,562 d.	Lal	1925
Thick susp., dried in vacuo over H_2SO_4, R.T.	Innoc. to blood or serum agar. recov. .05–.005% 24–48 hrs.	Otten	1930
NaCl soln., R.T., dried in vacuo	Innoc. blood or serum agar. Viable mos.	Otten	1930
Saline, —78 C., freeze in CO_2	25%	Proom	1949
Broth, —78 C.	96.5%	Proom	1949
Broth, R.T.	32.2%	Proom	1949
Saline, —78 C.	42.3%	Proom	1949
S. paradysenteriae (Flexner)			
Bouillon	>1,044	Lal	1921
Mutton, agar slope	1,562 d.	Lal	1925
S. paradysenteriae (His Y)			
Mutton, agar slope	1,562 d.	Lal	1925
S. pseudodysentery			
Physiol. saline, R.T.	.....	Otten	1930

TABLE C20—(Continued)

The Survival of Shigella Species in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
S. spp.			
Alcohol, ether, chloroform, 37 C.	Few hrs.	Vaillard	1903
SOLID			
S. dysenteriae			
Crust of rye bread, R.T., placed on Drigalski med.	Viable 20 d.	Bachmann	1943
Crust of rye bread, R.T.	66 d.	Bachmann	1943
Solid substance (Y type)	5 d.	Tashiro	1932
S. paradysenteriae (Flexner)			
Crust of rye bread, R.T., placed on Drigalski med.	Viable 23 d.	Bachmann	1943
Crust of rye bread, —5 to —25 C.	Viable 45 d.	Bachmann	1943
S. sonnei			
Crust of rye bread, R.T.	Overgrown with spores of bacillus	Bachmann	1943
GENERAL			
S. dysenteriae			
48 hr. cult., 37 C.	13 d.	Bamberger	1936
48 hr. cult., 22 C.	15 d.	Bamberger	1936
48 hr. cult., 10 C.	24 d.	Bamberger	1936
72 hr. cult., 37 C.	13 d.	Bamberger	1936
72 hr. cult., 22 C.	16 d.	Bamberger	1936
72 hr. cult., 10 C.	24 d.	Bamberger	1936
Bouillon, strong sun	<30 min.	Bamberger	1936
S. dysenteriae			
.....	10 d.	Kister	1928
Dark, R.T.	920 d.	Lal	1925
Stored 37 C.	4.5% survived 4 yrs.	Stamp	1947
Excess lime, pH 9.5–10	>300 min.	Wattie	1943
Excess lime, pH 10–10.5	>300 min.	Wattie	1943
Excess lime, pH 10.5–11	180 min.	Wattie	1943
Excess lime, pH 11–11.5	75 min.	Wattie	1943
S. equuli			
Cult., R.T., pH 7.4, stored in dark	3–4 mos.	McCollum	1951
S. ambigua (Schmitz)			
Cult. in bouillon, strong sunlight	<40 min.	Bamberger	1936
S. kruse			
48 hr. cult., 37 C.	12 d.	Bamberger	1936
48 hr. cult., 22 C.	15 d.	Bamberger	1936
48 hr. cult., 12 C.	24 d.	Bamberger	1936
72 hr. cult., 37 C.	13 d.	Bamberger	1936
72 hr. cult., 22 C.	15 d.	Bamberger	1936
72 hr. cult., 12 C.	24 d.	Bamberger	1936
Bouillon, strong sun	<30 min.	Bamberger	1936
S. paradysenteriae (Flexner)			
Bouillon, strong sun	<60 min.	Bamberger	1936
R.T., dark	1,049 d.	Lal	1925

TABLE C21

The Survival of Staphylococcus Species in Culture

Factor(s)	Survival	Reference	
GENERAL			
S. albus			
Stock lab. cult., sealed, R.T.	11–12 yrs.	Ahuja	1935
.....	523 d.	Lal	1921
Material obtained from sputum, antrum, nose, skin, throat, urine, uterus and put in broth and adjusted with HCl and NaOH. Of 12 strains used 7 viable at pH 2.6; 4 at pH 5.0; 1 at pH 8; 8 at pH 10 for 24 h.		Hall	1921
S. aureus			
.....	524 d. (Bombay str.)	Lal	1921
.....	>692 d.	Lal	1921
Dark, R.T.	657 d.	Lal	1925
Material obtained from boil, abscess, sycosis, spinal fluid, in broth	11 strs. viable at pH 2.6; 6 at pH 8; 13 at pH 10 in 24 h.	Hall	1921
S. citreus			
.....	523 d.	Lal	1921
S. spp.			
Desiccation	30 yrs.	Fasquelle	1950
60–100 C., in broth and dist. water, dried on collodion	Cell membrane separated	Ruska	1941
Very resistant to drying		Rhodes	1950
MICROCOCCUS SPECIES			
GENERAL			
M. melitensis			
.....	523 d.	Lal	1921
M. neoformans			
.....	523 d.	Lal	1921
M. pyogenes			
Sensitive to .85% NaCl		Zeug	1920
SOLID			
M. aurococcus			
Agar covered with sterile 10% cane sugar soln. and holding at 10 C.	8 mos.	Keith	1913
M. candicans			
Nutrient gel, 22 C.	No change, 8 d.	Ransome	1901
M. catarrhalis			
20 C.	3 mos.	Worth	1919
DRIED, GENERAL			
S. aureus			
Dry ice and ether	Wks.	Paul	1907
Liquid air	125 d.	Paul	1907
Dried on small stones in vacuo at low temp.	Recov. 40%	Paul	1907
Vacuum dried, —195 C.	16–22 d.	Shattock	1912
Air dried	16–22 d.	Shattock	1912
S. spp.			
Dried on garnets, R.T.	Innoc. 90,000, recov. 300, 32 d.	Paul	1909

TABLE C21—(Continued)

The Survival of Staphylococcus Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL, Continued			
Dried on garnets, ice box	Innoc. 88,800, recov. 550, 32 d.	Paul	1909
Dried on garnets, temp. of liquid air	Innoc. 65,990, recov. 67,900, 32 d.	Paul	1909
Dried	4–5 yrs.	Proom	1949
LIQUID			
S. albus			
.85% NaCl, —252 C.	50 h.	Kadisch	1931
S. aureus			
Saline susp., 6–11 C., R.H., 70–85%, CO_2 5–4%	70% destruct., >8 d.	Arai	1931
High pressure, R.T., 3000 atmospheres	45 min.	Basset	1932
Broth	15 d.	Bellelli	1928
Pus culture, R.T., sealed glass tubes	2½–3½ yrs.	Belin	1933
Dextrose, bouillon cult., freezing, 48 h., vacuum desic.	54 d.	Hammer	1911
Bouillon susp., R.T., in cellar, fine drops	35 d.	Kirstein	1902
Pure culture	28 d.	Kirstein	1902
Liquid air, —185 C.	No impaired vitality 20 h.	MacFayden	1899
Liquid air	6 mos.	MacFayden	1900
Liquid H_2, —252 C.	10 h.	MacFayden	1900
Physiol. NaCl soln. R.T., dried in vacuo over H_2SO_4	Viable for mos.	Otten	1930
Serum, 37 C.	Up to 11 d.	Panisset	1925
Met. violet	Recov. 0, 1 min.	Philibert	1926
Saline, —78 C., freeze in CO_2 ice	100%	Proom	1949
Broth, freeze in CO_2	100%	Proom	1949
37 C.	Viable 2 yrs.	Rhodes	1950
SOLID			
S. spp.			
Nutrient agar, below 0 C.	No growth	Haines	1934

TABLE C22

The Survival of Streptococcus Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
S. species			
Dried	7 yrs.	Lal	1948
Dried	4–5 yrs.	Proom	1949
Dried	⅔ of original, 97 d.	Stark	1931

TABLE C22—(Continued)

The Survival of Streptococcus Species in Culture

Factor(s)	Survival	Reference	
DRIED, LYOPHILIZED			
S. hemolyticus			
Dried 12 h.	51 mos.	Swift	1921
Frozen and dried	Many yrs.	Swift	1937
LIQUID			
S. hemolyticus			
Citrate, gelatin, R.T.	>3 d.	Meleney	1924
Lockes gelatin, 37 C.	>12 h.	Meleney	1924
Bacteria indifferent to osmotic pressure. Bivalent and univalent salts antagonize each other at certain levels.		Meleney	1924
Susp. in broth or saliva in dry air	Highly suscept. to triethylene glycol vapor after 5 h. desiccat.	Robertson	1951
Susp. in broth or saliva in dry air, low R.H.	Slower rate of killing	Robertson	1951
Veal infusion broth, 22 C.	At least 60 d. (alpha)	Surgalla	1944
Veal infusion broth, 37 C.	At least 30 d. (alpha)	Surgalla	1944
S. spp.			
Egg white, 50 cc.	4 h.	Belin	1933
Peptone 1/20	3 h.	Belin	1933
Alanine 1/20	5 h.	Belin	1933
Glycochol 1/100	13.5 h.	Belin	1933
Leucine 1/100	13.5 h.	Belin	1933
Physiol. NaCl, R.T., absolute drying in vacuo	Viable months	Otten	1930
Sprayed culture	All organisms settled in 48 hrs.	Phelps	1939
Sensitized with methyl violet	Recov. 0, 30 min.	Philibert	1926
Brain tissue, glycerol 50%	>303 d.	Rhoads	1929
S. salivarius			
Serum broth, 17 C., R.H. 77%, ozone .6–4 PPM.	>99% killed	Elford	1942
GENERAL			
S. agalactiae			
	Mos.	Watts	1941
R.H. 0	3 yrs.	Watts	1945
R.H. 75%	8 wks.	Watts	1945
R.H. 55%	26 wks.	Watts	1945
R.H. 25%	156 wks.	Watts	1945
R.H. 10%	156 wks.	Watts	1945
S. aureus			
Stored at 37 C.	.....	Stamp	1947
S. fecalis			
Stock lab. cult., sealed test tube, R.T.	11–12 yrs.	Ahuja	1935
S. hemolyticus			
Capillary tubes, —17 to —18 C.	2 wks.	Citovicz	1928
S. hemolyticus			
42 C.	Can stand numerous transfers	Spencer	1942

TABLE C22—(Continued)

The Survival of Streptococcus Species in Culture

Factor(s)	Survival	Reference	
GENERAL, Continued			
S. lactis			
Young cells, 1 C.	Innoc. 28,000,000, recov. 620,000, 62 d.	Sherman	1942
Mature cells, 1 C.	Innoc. 350,000,000, recov. <1, 51 d.	Sherman	1942
S. spp.			
R.H. 70–90%	Rapidly killed	Elford	1945
Death rate of S. spp. decreased in certain carbohydrates when desiccated. Using progressively dust, water, xylose, saline, tryptophane, salicin, glucose and sucrose the per cent death rate decreased from 66.8 to 5.0 desiccated compared with 81.6 to 88.0 for the same fluid controls.		Heller	1941
Varies in resistance to methylene blue		Louros	1923
60–100 C., 4–24 h. broth or agar cult.	Plasmolysis	Ruska	1941

TABLE C23

The Survival of Treponema Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
T. pallidum			
40 c.	2 hrs.	Besseman	1930
42 c.	1 hr.	Besseman	1930
Dried	1–2 d.	Landsteiner	1906
Dried in vacuo, R.T.	No movement, 4 d.	Zurhelle	1927
Under paraffin, liquid, R.T.	Movements 3 d, 17 hr.	Zurhelle	1927
Under paraffin, ice box	Movements 5–7 d.	Zurhelle	1927
Dried in vacuo, R.T.	No movement 68 h.–4 d.	Zurhelle	1927
Dried in Petri dish, R.T.	Still viable 65 hrs.	Zurhelle	1927
DRIED, LYOPHILIZED			
T. pallidum			
7 mo. cult., dried from frozen state. Stored 8 C.	168 hrs.	Hampp	1947
Rabbit testes in infusion broth,			
—78 C.	3 yrs.	Turner	1939
—10 C.	<2 mos.	Turner	1938
—20 C.	<2 mos.	Turner	1938
—78 C.	1 yr.	Turner	1938
—78 C.	1 yr.	Turner	1938
T. pertenue			
Rabbit testes in infusion broth,			
—78 C.	3 yrs.	Turner	1939
—78 C.	1 yr.	Turner	1939

TABLE C22—(Continued)

The Survival of Treponema Species in Culture

Factor(s)	Survival	Reference	
LIQUID			
T. hispanicum			
Blood of G.P., 14–20 C.	29 samples; 25 virulent 2–33 d.	Sergent	1938
Blood of G.P., 0 C.	6 samples; 0 virulent 33–60 d.	Sergent	1938
Blood of G.P., 0 C.	20 samples; 18 virulent 2d–7 wks.	Sergent	1938
Blood of G.P., 10 C.	1 d.	Sergent	1938
Choriollantoic memb. of 150 h. chick embryos.	<4 h.	Sterzi	1939
T. pallidum			
Saline susp. 45 C.	Killed 7–10 min.	Bronfenbrenner	1913
10% rabbit serum, —10 C.	>15 d.	Hindle	1934
½ inactive dog serum, ½ physiol salt soln., 20.4–3.65 abs.	4 h.	Jahnel	1938
Above at 1.7 abs.	2½ h.	Jahnel	1938
Serum exudate from chancre R.T.	121 d.	Lacy	1921
Saline susp. rabbit testes, R.T.	58 d.	Lacy	1921
Brewers fluid, thioglycollate below 37 C. incub. with rabbit testes	Improved survival	Nelson	1948
Expos. to 5% CO_2 with isotonic PO_4 buffer, pH 7.0	Prolonged survival	Nelson	1948
T. pallidum			
Isopropylidene with TPS factor, 147–148 C.	Prolonged survival	Rice	1951
Rabbit plasma, 5 C.	6 d.	Selbie	1943
Chorioallantoic memb. of 150 h. chick embryos.	<4 h.	Sterzie	1939
Physiol NaCl and human serum under vaseline, R.T.	Motionless, 5 d.	Zurhelle	1927
SOLID			
T. spp.			
Deep tubes of agar anaerobically under paraffin oil at R.T. and 37 C. in dark was not satisfactory.		Rosebury	1950
GENERAL			
T. pallidum			
Culture, 39 C.	5 h.	Boak	1933
Culture, 40 C.	3 h.	Boak	1933
Culture, 41 C.	2 h.	Boak	1933
Culture, 41.5 C.	1 h.	Boak	1933
—16 C.	3 mos.	Kissmeyer	1928
2 C.	Some days	Kissmeyer	1928
Cult. between 0 and 5 C.	>101 h.	Krantz	1923
10 C.	Lost power of producing lesion, 3 h.	Landsteiner	1906
Ice chest	24 h.	Landsteiner	1906
48 C.	30 min.	Landsteiner	1906

TABLE C22—(Continued)

The Survival of Treponema Species in Culture

Factor(s)	Survival	Reference	
GENERAL, Continued			
37 and 0 C.	4 h.	Miyao	1930
Syphilitic matl., 10 C.	3 h.	Neisser	1911
Syphilitic matl., ice chest	24 h.	Neisser	1911
Syphilitic matl., 48 C.	30 min.	Neisser	1911
Crystalline bovine alb., Na pyruvate, inorg. PO_4 buffer, glutathione cysteine, vitamins and $NaHCO_3$, 30 C., under 5% CO_2—95% N_2	8–10 d.	Nelson	1948
Culture of rabbit testes	10 d.	Perry	1948
Stored in solid CO_2	5–20 mos.	Rosebury	1950
Chick embryos, 35 C.	>8 d.	Wile	1941
Aerobic, damp chambers, R.T.	4 d.	Zurhelle	1927
Aerobic, damp chambers, 37 C.	48 h.	Zurhelle	1927
Aerobic damp chambers, ice box	Still viable 3 d., 17 h.	Zurhelle	1927
T. pertenue			
60 C.	15 min.	Marchoux	1938
37 C.	2 h.	Miyao	1930
0 C.	<15 min.	Miyao	1930
T. spp.			
Temp. ranging from that of liquid O_2 to that of liquid He	Survived	Luyet	1938

TABLE C24

The Survival of Vibrio Species in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
V. comma			
Peptone, water cult., dried in vacuo over P_2O_5. R.T. Sealed glass tubes	4 yrs.	Campbell-Renton	1942
(Schillong 653)	Orig. count, 9 X 10^6 After 4 yrs., 240	Campbell-Renton	1942
(Rangoon R)	Orig. count, 6 X 10^6 After 4 yrs., 50	Campbell-Renton	1942
(Schillong 1077)	Orig. count, 9 X 10^6 After 4 yrs., 50	Campbell-Renton	1942
(Schillong 610 R)	Orig. count, 125 X 10^7 After 4 yrs., 0	Campbell-Renton	1942
Dried	4–5 yrs.	Proom	1949
V. foetus			
Dried	3–4 yrs.	Proom	1949
LIQUID			
V. comma			
Sterile sat. soln. NaCl in water	<1 d.	Arguelles	1927
Fish extract, sterile	125 d.	Arguelles	1927

TABLE C24—(Continued)

The Survival of Vibrio Species in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Bouillon cult. with cont. freeze —10 to —16 C.	57 d.	Brehme	1901
Bouillon, —8 to —18 C.	Perished in 5 d.	Brehme	1901
Peptone, water cult., R.T., dried in vacuo, sealed tubes over P_2O_5	4 yrs.	Campbell-Renton	1942
0.3 g. NaCl and 10 cc. agar 38 C. (El tor)	Normal and invol. forms.	Eisler	1909
38 C.; .3 gm. NaCl	Invol. and normal forms	Eisler	1909
0.3 gm. NaCl and 0.05 gm. $Ca(NO_3)_2$	Normal forms	Eisler	1909
0.3 gms. NaCl and 0.1 g. $Ca(NO_3)_2$	Invol. and normal forms	Eisler	1909
Meat infusion	4–5 wks.	Hesse	1889
Ham broth	4–5 wks.	Hesse	1889
Bouillon susp., R.T.	10 h.	Kirstein	1902
Bouillon cult., 106 d. old			
dried in air	2 d.	Kitasato	1889
dried in exicator	3 d.	Kitasato	1889
Bouillon cult., 15 d. old			
dried in air	3 d.	Kitasato	1889
dried in exicator	3 d.	Kitasato	1889
Bouillon cult., 1 d. old			
dried in air	30 h.	Kitasato	1889
dried in exicator	40 h.	Kitasato	1889
Bouillon cult., 40 h. old			
dried in air	40 h.	Kitasato	1889
dried in exicator	40 h.	Kitasato	1889
V. comma			
.....	>894 d.	Lal	1921
..... (Bombay str.)	>657 d.	Lal	1921
.... (Lister str.)	>658 d.	Lal	1921
Liquid air, —185 C.	No impaired viabil. 20 h.	MacFayden	1899
Peptone broth, liquid air, —252 C.	No impaired vitality 10 h.	MacFayden	1900
Broth emulsion with unster. milk, liquid air	No impaired vitality 7 d.	MacFayden	1900
Liquid H_2, —252 C.	10 h.	MacFayden	1900
Thick susp., R.T., dried in vacuo over H_2SO_4	1 out of 100,000 survive 24–48 h. after innoc. into blood or serum	Otten	1930
Physiol. NaCl, R.T., abs. drying in vacuo	Innoc. blood or agar cult., viabl mos.	Otten	1930
Saline	12%	Proom	1949
Broth	47%	Proom	1949
Broth, R.T.	5.5%	Proom	1949
Saline, —78 C.	4.7%	Proom	1949
1/5,000 peptone with salt	3 d.	Read	1939
Ster. salt, water, ice 0.5–7.0 C.	6–7 d.	Renk	1893

TABLE C24—(Continued)

The Survival of Vibrio Species in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Ster. salt, water, ice 0.5–7.0 C.	Innoc. 1,483,000/cc. recov. 62,445/cc. after 24 h.	Renk	1893
Beef bouillon in cotton stoppered test tube	Viable and path. 5 wks.	Skidmore	1932
30% sat. NaCl	24 h.	Tohyama	1925
1 and 5% soln., 5th class table salt	4 d.	Tohyama	1925
Peptone water	11 d.	Tohyama	1925
Bouillon	26 d.	Tohyama	1925
NaCl 20 and 25%, —70 C.	24 h.	Tohyama	1930
Peptone water, —60 C.	Innoc. 40,000/cc., recov. 0, 1 mo.	Tohyama	1930
Bouillon, —60 C.	Innoc. 40,000/cc., recov. 0, 40 d.	Tohyama	1930
1 to 25% saline	Survived 3 d.	Tohyama	1930
20% NaCl, —70 C.	Innoc. 390,000/cc., recov. 0, 24 h.	Tohyama	1930
Physiol. NaCl, —70 C.	48 h.	Tohyama	1930
Peptone water, pH 7.9	>108 d.	Tohyama	1930
Bouillon, pH 7.9	>108 d.	Tohyama	1930
Peptone, 5–6.5 C.	21 d.	Weiss	1894
Bouillon, 22–25 C., dark	16 d.	Weiss	1894
SOLID			
V. comma			
10 cc. agar, 38 C., 0.46 gm. NaCl	Many invol. forms	Eisler	1909
V. comma			
10 cc. agar, 38 C., plus			
.46 gm. NaCl and .05 $Ca(NO_3)_2$	Dispersed invol. forms	Eisler	1909
.4 gm. NaCl and .1 gm $Ca(NO_3)_2$	Normal	Eisler	1909
0.1 gm. LiCl	Many involv. forms	Eisler	1909
0.1 g. LiCl and .05 g. $Ca(NO_3)_2$	Many normal, some invol.	Eisler	1909
.05 g. $Mg(NO_3)_2$	Invol. and normal	Eisler	1909
.05 g. $Mg(NO_3)_2$ and lg. NaCl	Many normal, few invol.	Eisler	1909
Thiol., 26 C.,pH 6.8	At least 150 d., max. growth 4 d.	Huddleson	1949
Beef peptone gelatine cult.			
—12.5 C., indoors	Lived 2 wks. after	Kasanky	1895
—31.8 C., outdoors	Lived 20 d.	Kasanky	1895
—30 to —31.8 C.	Viable 114 d.	Kasanky	1895
Gelatine cult., 102 d. old			
Dried in air	2 d.	Kitasato	1889
Dried in air	30 h.	Kitasato	1889
Dried in exicator	5 d.	Kitasato	1889
Dried in exicator	40 h.	Kitasato	1889
Gelatine cult., 12 d. old			
Dried in air	3 d.	Kitasato	1889
Dried in exicator	3 d.	Kitasato	1889
Gelatine cult., 4 d. old			
Dried in air	4 d.	Kitasato	1889

TABLE C24—(Continued)

The Survival of Vibrio Species in Culture

Factor(s)	Survival	Reference	
SOLID, Continued			
Dried in exicator	5 d.	Kitasato	1889
Agar cult., 20–22 C., 14 mos. old, 10 d. in incubator			
Dried in air	4 d.	Kitasato	1889
Dried in exicator	7 d.	Kitasato	1889
Agar cult., 20–22 C., 50 d. old, 12 d. in incubator			
Dried in air	4 d.	Kitasato	1889
Dried in exicator	11 d.	Kitasato	1889
Agar cult., 20–22 C., 9 d. old, 1 d. in incubator			
Dried in air	4 d.	Kitasato	1889
Dried in exicator	9 d.	Kitasato	1889
Agar cult., 20–22 C., 1 d. old, 1 d. in incubator			
Dried in air	3 d	Kitasato	1889
Dried in exicator	11 d.	Kitasato	1889
Potato cult., 17 d. old, 8 d. in incubator			
Dried in air	2 d.	Kitasato	1889
Dried in exicator	5 d.	Kitasato	1889
Potato cult., 8 d. old, 8 d. in incubator			
Dried in air	4 d.	Kitasato	1889
Dried in exicator	5 d.	Kitasato	1889
V. comma			
Gelatine agar, liquid air, —190 C.	No impaired vitality 7 d.	MacFayden	1900
Agar slant	42 d.	Tohyama	1925
GENERAL			
V. comma			
Stock lab. cult., R.T., sealed	Failed to show growth after 12–18 yrs.	Ahuja	1935
—40 F.	425 d.	Cole	1951
45 F., thawed and held 61–105 d.	Lasting immunity when used with hog cholera antisera	Cole	1951
—5.5 to —8 C.	Recov. 0, 10 d.	Finkelburg	1901
..... (Bombay str.)	>524 d.	Lal	1921
..... (Hog cholera)	>657 d.	Lal	1921
20 C. exposed to polarized light	Innoc. 1/100,000 diln. Recov. 121, 13 hrs.	Lal	1926
24 C., exposed to unpolarized light	Innoc. 1/100,000 diln. Recov. 17, 30 h.	Lal	1926
105 C.	Living	Lal	1923
110 C.	Dead	Lal	1923
Dark, R.T.	1,044 d.	Lal	1923
—21 C.	1 mo.	Rapschewski	1901
—15 C.	>1 mo.	Rapschewski	1901
37 C.	Dead at 2 yrs.	Rhodes	1950
Stored at 37 C.	0% survive after 4 yrs.	Stamp	1947
Lives more than 1 mo. when lowest temp. is —32.5 C. and repeated freezing and thawing has not influence on vitality.		Wuknow	1893

TABLE C25

The Survival of Viruses in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
Allantoid			
Dried and ppt. at 4 C.	6 mo.	Fasquelle	1950
Aphteuse			
Dried and left at 37 C.	2 yrs.	Fasquelle	1950
Herpes febrilis			
Ice box dried in vacuum over H_2SO_4 with reduced press., —5 C., Recov. in Lockes soln.	>1 yr.	Hawkins	1929
Herpetique			
Dried and left at 37 C.	2 mo.	Fasquelle	1950
Hoof and Mouth			
Dried with 0.5 g. P_2O_5 at 70 C. in vacuo	2½ hrs.	Sichert-Modrow	1930
Lymph, 122 C., dried with 0.5 g. P_2O_5 in vacuo	3 min.	Sichert-Modrow	1930
Dried lymph of G.P., 52 C. in atm. of P_2O_5	Still infectious, 14 h.	Sichert-Modrow	1930
Dried lymph of G.P., 52 C. in atm. of P_2O_5 R.T.	10 d.	Sichert-Modrow	1930
Influenza (Melbourne str.)			
Dried talc	30 min.	Parker	1944
Polio, (Aycock str.)			
Heat 30 min. at 50 C.	Remained infective	Shaughnessy	1930
Heat 30 min. at 52.5 C.	Non-infective	Shaughnessy	1930
Smallpox			
37 C., dried	80 d.	Hornibrook	1951
4–6 C., dried	24 h.	Hornibrook	1951
Tobacco mosaic			
Desiccated	Many yrs.	Stakman	1942
Vaccinia (testicular str.)			
Dried, 4 C.	12–18 mos.	Noguchi	1918
Dried	229 d.	Paschen	1908
DRIED, LYOPHILIZED			
Foot and mouth			
Dried in vacuo and refrig.	93 d.	Lepine	1937
Herpes			
0.5 fresh brain emuls. with Lockes soln.	4 wks.	Rivers	1927
Influenza			
Lyophilized	Infect. for ferret 14 mos.	Horsfall	1940
Infusion broth, rabbit testis, —78 C.	3 yrs.	Turner	1939
Mouse lung, 10% plain broth, —78 C.	6 mos.	Turner	1939
Laryngotracheitis (fowl)			
Lyophil. and stored 4 C.	3 yrs.	Hoffstadt	1946
Lymphogranuloma inguinale			
Infusion broth, rabbit testes, —78 C.	10 mos.	Turner	1939
Lymphocytic choriomeningitis			
Frozen, dried, 5 C.	378 d.	Wooley	1939

TABLE C25—(Continued)

The Survival of Viruses in Culture

Factor(s)	Survival	Reference	
DRIED, LYOPHILIZED, Continued			
Meningopneumonitis			
Infusion broth, rabbit testes, —78 C.	3 yrs.	Turner	1939
St. Louis encephalitis			
Frozen, dried, 5 C.	833 d.	Wooley	1939
Vaccinia			
Frozen, dried	mos.	Wooley	1939
Lockes soln., —185 C.			
Frozen and thawed 22X	No reaction, rabbit skin	Rivers	1927
Lyophil apparatus appears to be useful also in maintaining the viability of virus strains without continuous animal passage. (10 mos.)		Flosdorf	1935
LIQUID			
Coxsackie			
Susp., 53–55 C.	30 min.	Robinson	1950
Susp., pH 2.3–9.4	1 d.	Robinson	1950
Susp., pH 4.8	7 d.	Robinson	1950
Enteritis			
PO_4 buffer, pH 7	<20 d.	Gallo	1948
1/1,000 dil.	Killed 75% of mice	Gallo	1948
1/10,000 dil.	Killed 25% of mice	Gallo	1948
1/100,000 dil.	None killed	Gallo	1948
Equine encephalitis			
Acidic saline with agitation, bubbling, gases and shaking, pH 6.4	Rapidly inactivated	McLimans	1947
Foot and mouth			
GP blood 37 C., citrated	4.9 d.	Brooksby	1948
GP blood 37 C., defibrinate	2.1 d.	Brooksby	1948
M/45 buff. PO_4 soln., OC	Inoc. dil. 1/100,000	Galloway	1936
pH 7.6, purified	Recov. dil. 1/100, 97 d.	Galloway	1936
pH 7.6, unpurified	Inoc. dil. 1/100,000	Galloway	1936
	Recov. dil. 1/1,000, 126 d.	Galloway	1936
Saline, 37 C.	24 hrs.	Lepine	1937
50% glycerine	174 d.	Lepine	1937
GP lymph., pH 7.5 in PO_4 buffer	Still infectious, 2 yrs. 20 d.	Sichert-Modrow	1930
Ammonia brine, repeated freezing	Did not destroy, 124 d.	Stockman	1926
Fresh GP lymph, 2–7 C.	Retained virulence 190 d.	Stockman	1926
Herpes			
20% susp. of infected rabbit brain in buff. physiol. saline, 37C	100 hrs.	Boak	1940
20% susp. of infected rabbit brain in buff. physiol. saline, 41.5 C.	70–80 hrs. (Frank str.)	Boak	1940
20% susp. of infected rabbit brain in buff. physiol. saline, 41.5 C.	30 hrs. (Go str.)	Boak	1940
Fresh normal rabbit serum with UV radiation	10 min.	Gundersen	1932

TABLE C25—(Continued)

The Survival of Viruses in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Tissue cult., 40.2 C.	Did not survive	Thompson	1942
Influenza			
Single heat, chorioallantoic fluid (PR 8 unadapted) 60 C.	<5 min.	Jones	1945
Single heat, chorioallantoic fluid, (PR 8 Heat adapted) 60 C.	<5 min.	Jones	1945
Single heat, chorioallantoic fluid, (PR 8 Heat adapted) 56 C.	>45 to <60 min.	Jones	1945
Mouse brain susp. with rabbit serum, —20 to —30 C. (A and D)	<6 mos.	Olitsky	1949
Saline and horse serum pH 3.05	1 h.	Stock	1940
Saline and horse serum pH 4.04	1–20 h.	Stock	1940
Saline and horse serum pH 4.99	48 h.	Stock	1940
Saline and horse serum pH 5.35	48 h.	Stock	1940
Saline and horse serum pH 5.98	>72 h.	Stock	1940
Saline and horse serum pH 6.98	72 h.	Stock	1940
Saline and horse serum pH 7.0	72 h.	Stock	1940
Saline and horse serum pH 5.0	1 h.	Stock	1940
Saline and horse serum pH 7.5 with .001 m. oleic acid	90 min.	Stock	1940
50% glycerine	3–4 wks.	Wilson	1919
Japanese B. encephalitis			
Mouse brain susp. with rabbit serum, —20 to —30 C.	6 mos.	Olitsky	1949
Lymphogranuloma inguinale			
Aqueous susp.	40 hrs.	Latarjet	1951
Measles			
50% glycerine	3 mos.	VanRooyen	1940
Mumps			
Mouse brain susp. with rabbit serum, —20 to —30 C.	<6 mos.	Olitsky	1949
Chorio-allantoic fluid, 4 C., pH 6.5–7	Most stable	Weil	1948
Chorio-allantoic fluid, 4 C., pH above 7.9	Rapidly killed	Weil	1948
Chorio-allantoic fluid, 4 C., pH 5.8–8	99% intact., 4 wks.	Weil	1948
Penicillin and streptomycin	No inact. effect 14–28 d.	Weil	1948
Chorio-allantoic fluid, 37.5 C.	Greatest and most rapid increase, 7 d.	Weil	1948

TABLE C25—(Continued)

The Survival of Viruses in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Myxoma			
Tissue cult., 42.2 C.	Did not survive	Thompson	1942
Neurotropic			
Mouse brain susp. with rabbit serum, —20 to —30 C.	9 mos.	Olitsky	1949
Newcastle			
50% glycerine, R.T., pH 7.6	95 d.	Prier	1950
50% glycerine, R.T., 5 C., pH 7.6	353 d.	Prier	1950
Noguchi Str. (Vaccinia)			
Tissue cult., 45.1 C.	<4 d.	Thompson	1942
Poliomyelitis			
Glycerol	6 yrs.	Flexner	1917
Spinal cord in 50% glycerol, 4 C.	>25 mos.	Flexner	1914
Glycerol (M. A. str.)	>11 mos.	Flexner	1914
0.5% phenol	>14 d.	Flexner	1914
Filtered, 37 C.	20 d.	Flexner	1910
—2 to —4 C.	40 d.	Flexner	1910
4 C.	50 d.	Flexner	1910
45–50 C.	Killed after 30 min.	Flexner	1910
50% glycerol	>7 d.	Flexner	1910
50% glycerol —15 C.	2 yrs.	Flexner	1910
Aqueous susp.	10 d.	Latarjet	1951
Physiol. saline, 45–60 C. 30 min.	Becomes inactivated. The more dil. the susp. the less heat is req. to inactivate	Latarjet	1951
50% glycerol	8 yrs.	Rhoads	1929
100% glycerol, 18 C. 1st passage	Viable 59 d.	Romer	1910
50% glycerol, 3rd pass.	14–31 d.	Romer	1910
50% glycerine	Some mos.	Wilson	1919
Psittacosis			
Beef saline	29 d.	Rivers	1948
Rabies			
Brain susp. in glycerine in disintegrator 1 h.	47 d.	Barrat	1904
Susp. brain, liquid air	Still virulent 24 h.	Barrat	1904
Buffer soln., glycerol	15–17 wks.	Grycz	1949
Aqueous susp.	40 hrs.	Latarjet	1951
Undil. neutral glycerol, R.T.	Several wks.	Rivers	1948
Liquid air, —185 C.	3 mos.	McFayden	1900
Neutral glycerol, refrig.	Several mos.	Rivers	1948
Aqueous susp., 54–56 C.	1 h. or less	Rivers	1948
Aqueous susp., sub-freezing	1 or more yrs.	Rivers	1948
Streptobacillus virus			
Bouillon, 100 C., 48 h. cult., 1–100 dil. (Str. # 1776)	No growth	Bingel	1947

TABLE C25—(Continued)

The Survival of Viruses in Culture

Factor(s)	Survival	Reference	
LIQUID, Continued			
Vaccinia			
Glycerine susp. (28628, rabbit testicular)	33 d. and 6 hrs. after 61st transfer	Armstrong	1929
Chorio-allantoic memb.	8 hrs.	Buchbinder	1941
Glycerine, mouse brain, water	Inoc. .05 cc of 1:100 dil., Recov. after 24th passage, 10 mos.	Haagen	1939
Glycerine, mouse brain, water, freeze-dry temp.	1 yr. 9 mos.	Haagen	1939
Glycerine, mouse brain, refrig. temp.	Avirulent 1 yr.	Haagen	1939
Vaccinia			
Chorion & 1 drop calf lymph, Refrig. temp.			
(Str. 3, Breslau)	2 yrs.	Haagen	1939
(Str. 4, Dresden)	2 yrs.	Haagen	1939
Allantois of chick embryo, low temp., Present in glycerine, Ringers soln. from 1933–49	Capable of multiplication and still pathogenic after 15 yrs.	Lehmann	1949
Pure glycerol, 18 C.	5 d.	Noguchi	1918
Pure glycerol, 37 C.	24 h.	Noguchi	1918
Ringers soln. with .54 and 1% phenol and water	Still active 1 yr.	Noguchi	1918
Physiol saline, —13 C.	7.4% showed count over 30,000 after 4 yrs. 41% after 10 yrs.	Schartner	1939
Tissue cult., 45.1 C.	.....	Thompson	1942
Liquid air	15 min.	White	1901
Variola			
Saline soln., 35 C.	30 min.	Gordon	1925
Yellow fever			
Physiol. saline	Better than dist. water.	Bauer	1940
Aqueous susp.	10 d.	Latarjet	1951
Glycerol	8 mos.	Rivers	1948
SOLID			
Foot and mouth			
Congealed state	162 d.	Lepine	1937
Vaccinia			
Levinthal, blood agar, —13 C.	Recov. 11% after 4 yrs., 58.3% after 10 yrs.	Schartner	1939
GENERAL			
Colorado tick fever			
Ice compartment	3½ yrs.	Rivers	1948
Cow pox			
Culture, —70 C.	<108 h.	Pictet	1884
Culture, —130 C.	<20 h.	Pictet	1884
Dengue			
Stegomya, 22 C.	Up to 85 d.	Blanc	1929

TABLE C25—(Continued)

The Survival of Viruses in Culture

Factor(s)	Survival	Reference	
GENERAL, Continued			
Stegomya, 16.5 C.	Up to 85 d.	Blanc	1929
Stegomya, 22.5 C.	Up to 174 d.	Blanc	1929
Encephalitis			
40 C., pH 8.4 (St. Louis)	Still viable 3 wks.	Duffy	1946
pH 3.5–11.5 (Equine)	Greatest stability after 1 hr.	Finkelstein	1938
pH 7.5–8.5, (Equine)	Greatest stability after 1 wk.	Finkelstein	1938
20% saline with 10 cc. defibrin. rabbit blood temp. 29.4–32 C., high humidity (Jap. B.)	Transmission occurred by bite of Culex spp. mosq. in 8 days following blood meal	Hammon	1949
Encephalitis			
—20 C., glass tubes (Jap B.)	12 mos.	Melnick	1946
—20 C., corked tubes (Jap. B.)	1,000 fold loss	Melnick	1946
Filtered, 56 C. (St. Louis)	30 min.	Rivers	1948
pH 8.4–8.8 (St. Louis)	3 wks.	Rivers	1948
pH 7–10 (Jap B)	Inact. rapidly	Rivers	1948
Susp. 70 C. (West. equine)	10 min.	Rivers	1948
Filtrates, 60 C. (West equine)	10 min.	Rivers	1948
Fowl plague			
pH 6–9.9	Range of stability	Pyl	1938
Foot and mouth			
Quinea pig vesicle fluid, R.T.	3–6 mos.	Anonymous	.....
Herpes simplex			
—70 C.	>1 yr.	Rivers	1948
Influenza			
10% normal horse serum, shaken 24 h. (PR8 Str.)	Held titer better than 0.2% bovine albumin	Dick	1949
10% normal monkey serum, shaken 24 h. (French neurotropic)	Held titer better than 0.2% bovine albumin dil.	Dick	1949
Tissue culture, 60 C. (PR8)	>5 min., <7 min.	Jones	1945
Tissue culture, 55 C.	>30 min., <45 min.	Jones	1945
Tissue culture, 50 C.	>120 min., <180 min.	Jones	1945
Tissue culture, heat adapted, 60 C.	>5 min., <7 min.	Jones	1945
Tissue culture, heat adapted, 55 C.	>45 min., <60 min.	Jones	1945
Tissue culture, heat adapted, 50 C.	>180 min.	Jones	1945
After storage, (PR8) 25.5 C.	Unadapted 28 d., heat adapted 49 d.	Jones	1945
After storage, 37 C.	Unadapted 18 d., heat dapated 22 d.	Jones	1945
After storage, 41 C.	Unadapted 8 d., heat adapted 14 d.	Jones	1945
40 C. (Inf. A)	2–3 mos.	Rivers	1948

TABLE C25—(Continued)

The Survival of Viruses in Culture

Factor(s)	Survival	Reference	
GENERAL, Continued			
R.T.	6 wks.	Scherp	1938
0–40 C.	1 wk.	Scherp	1938
Inclusion conjunctivitis			
Refrigerated	Sev. days	Rivers	1948
Looping ill			
Mouse brain, 80 C.	30 sec.	Rivers	1948
Mouse brain, 60 C.	2 min.	Rivers	1948
Mouse brain, 56 C.	10 min.	Rivers	1948
Lymphogranuloma venerum			
37 C.	2–4 d.	Rivers	1948
56 C.	10 min.	Rivers	1948
70 to —30 C.	>1 yr.	Rivers	1948
Mumps			
—70 C.	10 mos.	Rivers	1948
Measles			
—72 to —35 C.	4 wks.	Rivers	1948
Freezing	25 hrs.	Van Rooyen	1940
Newcastle			
pH 5.5–7.5	.....	Elford	1948
Incub. 37 C., undil. amniotic fluid	Viable 126 d.	Olesiuk	1951
Papilloma			
Neutral to pH 4.2	Least loss of activity	Beard	1938
Weakly alkaline	Gradual loss of activ.	Beard	1938
On acid side of pH 7 the virus activity remains high until at pH between 2.9 & 3.3 it is lost suddenly. At pH 10.1 to 10.2 immediate inactiv. observed. (Shope virus.)		Wyckoff	1937
Poliomyelitis			
More effective when suspended in low pH.	(Armstrong mouse adapted)	Hammon	1941
Infective after heating at 55–58 C.	.....	Howitt	1930
Emulsion of hemoceles of flies and cockroaches	Produced paralysis in mice in 12 and 15 d. respectively	Hurlbut	1950
<38.5 C., 2800–3100 Å.	Complete destruction 1–30 min.	Jungeblut	1937
Berkefeld filter, 38 C.	7 d.	Landsteiner	1910
35 C.	2 hrs.	Leiner	1910
37 C.	21 d.	Levaditi	1913
—70 to —20 C.	No loss in infectivity 12 mos.	Melnick	1946
With E. histolytica	Settles out	Young	1949
50 C.	3 min.	Shaughnessy	1930
Pseudolymphocytic chorio meningitis			
Mouse brain, 56 C.	30 min.	Rivers	1948
Psittacosis			
Broth, 40 C.	Several wks.	Rivers	1948
—70 C.	>2 yrs.	Rivers	1948

TABLE C25—(Continued)

The Survival of Viruses in Culture

Factor(s)	Survival	Reference	
GENERAL, Continued			
Rabies			
Fats and lipids extracted from desiccated rabies vaccine with various solvents and injected into mice fail to give protection or provoke evidence of toxic response when given subdurally or intraperitoneally. When extracted at low temp. (—65 C.) with ether, the virus is not destroyed. After 1 yr. amount of living virus has not decreased.		Harris	1948
Rift valley fever virus			
Light, met. blue	40 min.	Rivers	1948
Rinderpest virus			
Leucocytes and spleen 4 C.			
Dried and ppt. with acetone	>2 mos.	Das	1949
Rinderpest virus			
Leucocytes and spleen 40 C.	>4 mos.	Das	1949
Dried over $CaCl_2$ in vacuo 37 C.	15 d.	Das	1949
Semlike forest virus			
60 C.	1 h.	Rivers	1948
Trachoma			
Refrigerate	1 wk.	Julianelle	1942
Vaccinia			
Ottens dry lymph, tropical temp. and R.H.	18 yrs.	Collier	1950
Gases at 4 C.	Retained virulence 3 wks.	Noguchi	1918
Gases at 37 C.	Became avirulent	Noguchi	1918
Pure O_2 or CO_2, 18 C.	Destroyed virus complete	Noguchi	1918
Sonic vibrations	>15 min. partial inact.	Rivers	1937
Yellow fever			
10% N. monkey serum shaken 24 h.	Held titer better than 0.2% bovine alb. dil.	Dick	1949
Desiccated, frozen	Many yrs.	Rivers	1948
Cold virus filtrates			
—76 C., dry ice	2 yrs.	Andrews	1949
—10 C.	27 d.	Andrews	1949
4 C.	3 d.	Andrews	1949

TABLE C26

The Survival of Yeasts, Molds and Fungi in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL			
Actinomyces			
Desiccated (wentii)	>1 yr.	Wehmer	1897
Desiccated (niger)	3 yrs.	Wehmer	1897
Desiccated (oryzae)	>4 yrs.	Wehmer	1897
Dried (spp.)	4–5 yrs.	Proom	1949
Brewers yeast			
Dried with plaster paris	10 mos.	Pasteur	1876
Saccharomyces pastorianus			
diffuse light	<3 yrs.	Kayser	1889

TABLE C26—(Continued)

The Survival of Yeasts, Molds and Fungi in Culture

Factor(s)	Survival	Reference	
DRIED, GENERAL, Continued			
Yeast, spp.			
More resistant in dry state than in moist		Kayser	1889
DRIED, LYOPHILIZED			
Ascomycetes			
Culture suspended in normal horse serum, quick frozen, dried in high vacuum and sealed quickly	0.2% survival in rate in 3 mos.	Atkin	1949
Saccharomyces cerevisiae			
Bouillon and beerwort, exp. 10–15 min. to U.V.	More time req. for gelatinized media	Gilles	1935
Frozen at —24 C. and thawed at 25 C.	28% killed	Stille	1943
Yeasts, spp.			
Lyophilized in vacuo	1 yr.	Dopter	1949
Lyophilized	2 yrs.	Wickerham	1946
LIQUID			
Aspergillus			
Nutrient media, —6 to —11 C.	4 d.	Bartetzko	1910
1% glucose, —12 C.	2 h.	Bartetzko	1910
Epidermophytes			
.85% NaCl, —20 to —30 C.	34 d.	Kadisch	1931
Saccharomyces			
Broth, salt soln., —13 to —15 C. (cerevisiae)	>160 wks.	Tanner	1928
Broth, —13 to —15 C. (ellipsoideus)	5 wks.	Tanner	1928
Salt soln., —13 to —15 C. (ellipsoideus)	18 wks.	Tanner	1928
Broth, —13 to —15 C. (maxicans)	>160 wks.	Tanner	1928
Salt soln., —13 to —15 C. (maxicans)	14 wks.	Tanner	1928
Broth, —13 to —15 C. (pastorianus)	58 wks.	Tanner	1928
Salt soln., —13 to —15 C. (pastorianus)	>160 wks.	Tanner	1928
Yeasts, spp.			
10% sucrose	Of 25 strs., 15 retained viability 10¼ yrs., 9 dead in 8¼ yrs.	Meissner	1911
Nutrient broth and fruit juices, —23.3 C.	3 yrs.	Tanner	1934
Yeasts, spp.			
10% sucrose in Freudenreich flasks	Many yrs.	Will	1909
.85% NaCl soln., —20 to —30 C.	>2 mos.	Kadisch	1931

TABLE C26—(Continued)

The Survival of Yeasts, Molds and Fungi in Culture

Factor(s)	Survival	Reference	
SOLID			
Absidia			
Potato dextrose agar, 7 C.	10 used, 8 viable, 2 yrs. 8 mos.	Hesseltine	1947
Actinomucor			
Potato dextrose agar, 7 C.	1 used, 1 viable, 2 yrs. 8 mos.	Hesseltine	1947
Alternaria			
Potato dextrose agar, 7 C.	3 used, 3 viable, 2 yrs. 8 mos.	Hesseltine	1947
Agar slant, —29 C.	>4 mos.	Bartram	1916
Ascophyta			
Agar slant, —29 C.	>4 mos.	Bartram	1916
Aspergillus			
Potato dextrose agar, 7 C.	98 used, 48 viable, 2 yrs. 8 mos.	Hesseltine	1947
3% gelatin, —10 to —13 C.	12 h.	Lindner	1915
Blastomyces			
Sabourauds dextrose agar, R.T., covered with mineral oil	20 mos.	Ajello	1951
Candida			
Sabourauds dextrose agar, R.T., covered with mineral oil	20 mos.	Ajello	1951
Cephalotheuum			
Agar slant, —29 C.	>4 mos.	Bartram	1916
Chartomium			
Potato dextrose agar, 7 C.	8 used, 7 viable, 2 yrs. 8 mos.	Hesseltine	1947
Circinella			
Potato dextrose agar, 7 C.	2 used, 2 viable, 2 yrs. 8 mos.	Hesseltine	1947
Coccidioides			
Sabourauds dextrose agar, R.T., covered with mineral oil	20 mos.	Ajello	1951
Cryptococcus			
Sabourauds dextrose agar, R.T., covered with mineral oil	20 mos.	Ajello	1951
Fusarium			
Potato dextrose agar, 7 C.	15 used, 11 viable, 2 yrs. 8 mos.	Hesseltine	1947
Mucor			
Agar or liquid Raulins med., —70 to —110 C.	2 h.	Chodat	1896
Mucor			
Potato dext. agar, 7 C.	59 used, 53 viable, 2 yrs., 8 mos.	Hesseltine	1947
Nocardia			
Sabourauds dext. agar, R.T. Cov. with mineral oil	20 mos.	Ajello	1951
Phycomyces			
Potato dext agar, 7 C.	3 used, 3 viable, 2 yrs., 8 mos.	Hesseltine	1947

TABLE C26—(Continued)

The Survival of Yeasts, Molds and Fungi in Culture

Factor(s)	Survival	Reference	
SOLID, Continued			
Penicillium			
Potato dext. agar, 7 C. (spp.)	2 yrs., 8 mos.	Hesseltine	1947
3% gelatin, —10 to —13 C. (glaucum)	12 h.	Lindner	1915
Rhizopus			
Potato dext. agar, 7 C.	17 used, 13 viable 2 yrs., 8 mos.	Hesseltine	1947
Saccharomyces cerevisiae			
Agar slants, —70 C.	1–8 d.	Karcher	1931
Syncephalastrum			
Potato dext. agar, 7 C.	2 used, 2 viable, 2 yrs. 8 mos.	Hesseltine	1947
Trichoderma			
Potato dext. agar, 7 C.	7 used, 7 viable 2 yrs. 8 mos.	Hesseltine	1947
Yeast, spp.			
Nutrient agar, O C.	Rapid growth, 5 d.	Haines	1934
GENERAL			
Aspergillus			
Dark, lab. temp. (flavus)	6 yrs.	McCrae	1923
Dark, lab. temp. (fumigatus)	10 yrs.	McCrae	1923
Dark, lab. temp. (glaucus)	16 yrs.	McCrae	1923
Mold, spp.			
Dark, lab. temp. (spores)	20 yrs.	McCrae	1923
Below freezing	Very few living, 16 mo.	Tanner	1931
Saccharomyces			
Subfreezing temp, Sucrose conc. >30–35%, pH 3.6–3.7	Retards destruction	McFarlane	1940
Streptothrix	420 d.	Lal	1921
Yeasts, spp.			
Incubator, 28 C.	<3 yrs.	Kayser	1889
Sub-freezing, —9.9 C. in agar slant	>1 yr.	Smart	1935
Found in frozen fruits, 15 F.	After 3 yrs.	Smart	1935
—15 C.	160 wks.	Tanner	1928
Yeasts, spp.			
Agar, 37 C.	5 mos.	Eberson	1920

SUMMARY OF ABBREVIATIONS USED IN TABLES

alk.	alkaline	F.	Degrees fahrenheit
avg.	average	fl.	fluid
C.	Degrees centigrade	G.P.	Guinea pig
Col.	Colonies	gel.	Gelatin
conc.	concentration	h., hrs.	hour or hours
cont'd, cont.	continued	inc.	increase
ct.	count	Inoc., Innoc.	Inoculate
cult.	culture	irrad.	irradiated
d., ds., das.	day or days	Lg.	Large
Desic.	Desiccate	max.	maximum
dil.	dilution	med.	medium

met.	methyl	soln., sol'n	solution
min.	minute or minutes	spp.	species
mos.	months	str.	strain
mult.	multiplied	susp., susp'n	suspension
org.	organism	T.B., tb	tuberculosis
path.	pathogenic	temp.	temperature
physiol.	physiological	U.V., U.v., UV	Ultra violet
ppm.	parts per million	wks.	weeks
ppt.	precipitate	X	times
R.H.	Relative humidity	yr., yrs.	year or years
R.T.	Room temperature	>	greater than
Recov.	Recovered	<	less than
refrig.	refrigeration	+	present; plus
sec.	second	0	none
sensit.	sensitization	—	minus

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THE PRESENTATION OF SPATIAL VECTORCARDIOGRAPHIC DATA ON A LINEAR TIME SCALE*

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Electrocardiographic leads reflect electrical activity of the heart projected on a variety of axes. This activity can also be presented as lissajous figures on cathode ray oscilloscopes (vectorcardiograms.) Such a presentation has the advantage of displaying the locus of activity in selected planes by combining linear projections in perfect time phase, but it is difficult to represent absolute temporal relations. The advantage of the vectorcardiogram can be retained, and an adequate representation of time achieved by presenting the spatial magnitude and two angular functions of the heart's electrical activity as separate traces on a linear time scale. Such a presentation has been suggested by McFee (1950) who also reported the construction of a computer for direct registration of such records from two Cartesian components. The possible advantages of this type of display have been noted by Frank (1953), and Sayer, Silberberg, and Durie (1955) have reported observations on linear time scale magnitude curves.

In the present studies linear time scale curves of spatial magnitude R, orientation in the frontal plane of the body τ, and orientation about an anteroposterior axis ϕ have been derived from electrocardiographic leads. Thirty-nine normal subjects and 18 subjects showing a variety of electrocardiographic abnormalities were studied. Two methods of electrode placement were employed on each subject. In one method the body was considered to be represented by an equilateral tetrahedron and appropriate electrode combinations to yield three Cartesian components were utilized. The other method of electrode placement was that suggested by McFee and Johnston (1954) to obtain horizontal and anteroposterior components of the heart's electrical activity and utilized lead V_F as the vertical component. With each lead system the Cartesian components were recorded simultaneously

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at a film speed of 100 mm. per second. Lissajous figure vectorcardiograms were also recorded on each subject.

Measurements of the magnitude of Cartesian components were made with a magnifying film reader which permitted measurements down to intervals of 0.003 second. Conversion to spherical coordinates was carried out by an IBM Card Program Computer and the values of R, θ and ϕ plotted as linear functions of time by an automatic plotter. θ was represented on a scale of 0 to 360° and ϕ on a scale of —90 to +90°. Examples of the linear time scale plots are shown in figure I.

The information in the electrocardiograms which is known to be clinically important was present in easily accessible form in the linear time scale plots. In addition these plots had features suggesting intrinsic merit in this method of presentation. The curves of R showed notching in the QRS portions which was not easily apparent from inspection of the electrocardiograms or lissajous figure vectorcardiograms. This notching was more common in records obtained with the lead system of McFee and Johnston and was most marked in the records of some subjects with myocardial infarctions.

The highly variable orientation of the QRS of normal lissajous figure vectorcardiograms was accurately reflected in the linear time scale plots, yet the curves of θ during this period were easily categorized into monotonic and two types of inflected functions. All linear time scale plots of ϕ from normal subjects were inflected curves and showed remarkable uniformity.

One of the most interesting features of vectorcardiographic data presented in the form described is its susceptibility to a variety of analytical methods. Since variations in the heart vector with time are completely described by this method of presentation, certain mathematical manipulations are likely to yield meaningful data, whereas similar operations on the Cartesian components or lissajous presentations are not. One such method of analysis has been employed in this study. The QRS portions of the R curves have been described by a series of numbers including pulse width, ratio of notch depth to maximal amplitude, ratio of QRS and T areas, and quantities analagous to kurtosis and skewness. Statistically significant differences in the width, kurtosis and skewness of normal magnitude curves and those

from patients with myocardial infarction were found with both electrode systems employed. A significant difference in the ratio of QRS and T areas between normal records and those showing infarction was also found in records obtained with the lead system of McFee and Johnston. These measurements illustrate the

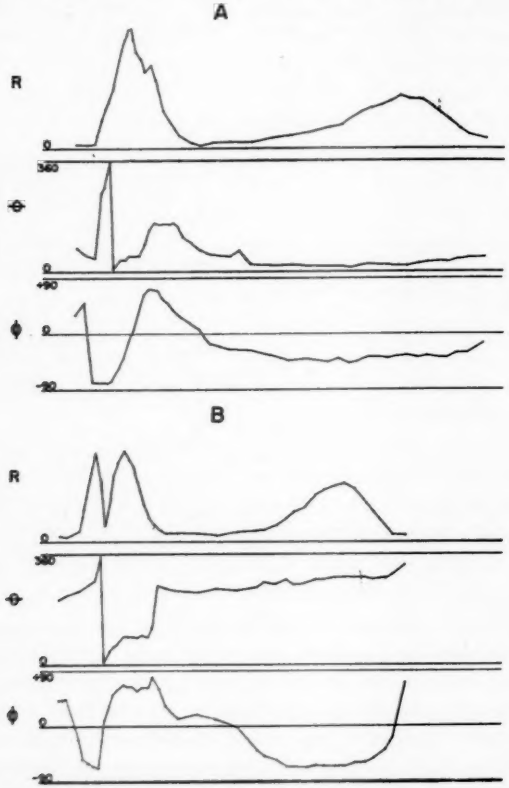

FIG. 1. Curves of spatial magnitude R, frontal plane angle $\div$, and orientation about an anteroposterior axis ϕ of electrical activity of the heart during the QRS and T portions of the ECG. A shows curves from a normal subject and B those from a case of posterior myocardial infarction. Both records were derived from ECG leads recorded with the electrode placement of McFee and Johnston. $\div$ is presented on a scale of 0 to 360° with the point representing these values being the left side of the horizontal component in the subject. Note that without the meaningless step from 360° to 0° both traces of $\div$ are monotonic in the QRS portions. ϕ is represented on a scale of —90° to +90° with the +90° axis directed posteriorly.

possibility of more precise analysis of curves reflecting electrical activity of the heart than has been possible previously.

Other promising manipulations which may be applied to vectorcardiographic data on a linear time scale include the simple derivatives of the curves and the vector derivatives. Analysis of the form factor of spatial magnitude curves has been reported by Sayer, Silberberg and Durie (1955). Considerable study will be necessary to define the most significant measurements.

It appears possible to achieve the direct registration of traces of the type described and a computer for this purpose has been designed.

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BRAIN MODELLING, A BRIEF DESCRIPTION OF STUDYING THE BRAIN ANATOMY BY RECONSTRUCTION*

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I. *Introduction*

An effective method of studying the structures and connections of the nervous system is to be obtained in brain modelling. In the process of constructing a three dimensional diagram of the brain, definite and comprehensive anatomical knowledge is incorporated with embryological, neurological and physiological evidences, a fact which serves both to encourage investigation on the part of the student and to elucidate lectures on the part of the teacher.

Since 1937, an elective course in brain modelling has been offered annually to the research fellows in the Montreal Neurological Institute. This course was introduced by Dr. Francis McNaughton after his visit to the laboratory of Adolph Meyer and L. Hausman (3,4) in Baltimore, and has been conducted under his direct supervision. A brief description of the course was given by Barr in 1946 (1), after which time numerous additions have been made to the model and to the method of construction. This paper, prompted by those who benefited from the course, strives to bring Barr's description up to date. It is the authors' hope that the details and diagrams supplied here will be helpful to those who wish to give a similar course, as well as to the scholars who undertake the study upon their own initiative.

If a student learns where the major structures are located by molding a three dimensional model in clay and wire, using normal fixed specimens, books and diagrams as his guide, he creates a lasting impression which will serve him well when he encoun-

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ters the living brain in pathological conditions. Building a model makes the student aware of his deficiencies, and brings to light the controversial issues because he is forced to defend his model against the onslaught of his teachers, fellow students, and conflicting statements in the literature.

II. *Materials*

1. *Gross and microscopic specimens.* While the information from the standard neuroanatomical textbooks is essential for building a "brain model," the actual dissection of a formalin fixed brain with an orange stick provides clearer indications of the location, relation, and connections of the various structures and the fiber tracts of the nervous system. It is found that Cunningham's Manual of Anatomy is most helpful for the dissection of a brain. The Department of Pathology is the chief source of the supply of the brain specimens.

Apart from the dissection, demonstration materials should be available in the laboratory. They are serial sections of 1/2 to 1 centimeter in thickness of brains in coronal, longitudinal, and horizontal planes. Demonstrations of regional structures, such as the internal capsule, the cingulum, the mamillo-thalamic tract, the extra and intra-medullary course of the trigeminal nerve, etc., are provided by the fellows who participate in the course.

Several well-dissected skulls are placed at the disposal of the workers in the laboratory. This is of paramount importance when the basal structures and the course of the cranial nerves are being studied.

Microscopic materials include sets of serial transverse sections of the brain stem stained by Weigert-Pal, Nissl, and Heidenhain technique, and a set of serial coronal sections of a right cerebral hemisphere stained alternately by Nissl and Heidenhain techniques.

The Weigert-Pal series of the brain stem sections are mounted on large slides suitable for lantern projection. Tracing of the stained fibers with a hand lens is further facilitated by putting the slides upon a large illuminated viewing box.

The alternately stained sets are used in the detailed study of the nuclear structures. The sections of the brain stem series are cut 20 microns in thickness; every 10 to 20 sections are stained

with cresyl violet, and the adjacent one by the Heidenhain method. The numbers of mounted slides refer to the sections of the specimen, thus the rostro-caudal measurement of a nucleus can be readily calculated. For example, the abducens nucleus is found in the slides from number 1500 to 1680. The ora-caudal measurement of the abducens is, therefore (1680–1500) × 20 = 3600 or 3.6 mm. Similarly, the measurements of all the nuclei can be worked out (Figs. I and II).

Actually, there are errors in these measurements since the 20 per cent to 40 per cent shrinking of the specimens during dehydration are variable. This discrepancy makes measurements from the slides considerably smaller than measurements from gross specimens.

The coronal sections of the right cerebral hemisphere are cut 25 microns thick, and every 24th and 25th sections are stained with cresyl violet and Heidenhain methods respectively. A working scale for the measurement and the relative position of the various thalamic nuclei is illustrated in figure III.

2. *Atlases.* To facilitate the use of the above slides, several atlases are provided.

A. Atlases of the brain stem sections from the Weigert-Pal preparations. Photographs, enlarged 4 times the size of the sections are made. Since the brain model is to be made 4 times the size of the adult brain, the size, shape, and relation of the structures in each photograph will correspond exactly to the model at various levels.

B. Atlas of the brain stem sections from the cresyl violet preparations. Photomicrographs with a magnification of 20 times are made from the series at selective levels. Cytoarchitectonic delineations of the cranial nerve nuclei and reticular nuclei in each photograph are mapped out with India ink. The brain stem atlas was prepared by one of the authors (K. E.) with the collaboration of Dr. Jerzy Olszewski, and the thalamic atlas was made by Dr. Anatole Dekaban.[1]

C. Atlas of the thalamus from sections of the right cerebral hemisphere prepared with cresyl violet and Heindenhain stains. Likewise, the representative sections are photomicrographed by

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a magnification of 25 times. Each thalamic nucleus in the photomicrograph is traced with India ink. All of the available textbooks of neuro-anatomy are made available to the student, and he is encouraged to use the library to seek original articles on controversial subjects.

The most helpful atlases which have become available in recent years are those of Riley (8), Ciba (Netter) (5), Hausman (2), Olszewski and Baxter (6), and Olszewski (7).

3. *Wooden framework:* The following items can be turned out by any carpenter shop and stocked in the laboratory. 1. Baseboard, 18 x 18 inches (Fig. VII). 2. Longitudinal section of corpus callosum and cerebellum. 3. Cross-section of the right cerebral hemisphere. 4. Cross-section of the right thalamus at the level of the red nucleus. 5. Cylindrical rods. These items are made 4 times their actual size in an average adult brain (Fig. IV).

4. *Plasticine.* Colored plasticine (modelling clay) is available in most art stores. For our purpose, the following colors are preferable: gray, dark red, light red, purple, brown, terra cotta, white, black, yellow, light blue, light green, dark green, and orange.

5. *Tools.* For general purpose, a few instruments consisting of stove bolts, a screw driver, wire, and a pair of pliers will suffice. However, for finer work, the following tools are valuable: 1. vise, 2. coping saw, 3. hack saw, 4. hammer, 5. file, 6. aluminum or steel rods 1/8″ or 1/4″, 7. aluminum or tin sheeting, 8. small angle irons, 9. wire mesh, 10. radio wires of different colored coatings, 11. piano wire or copper wire, 12. wire cutting pliers and sharp nosed pliers, 13. tin shears, 14. stove bolts 1/8″ x 1/4″ and 1/8″ x 3/4″, 15. wood screws 1/2″, 16. screw driver, 17. enamel paints of various colors, 18. spar varnish, 19. white lacquer, 20. turpentine, 21. India ink, 22. paint brushes, 23. pen points and staffs, 24. swab sticks, 25. orange sticks, 26. paint scraper, 27. sand paper, 28. calipers, 29. plastic wood, 30. glue, 31. scotch tape, and 32. rags.

III. *Method of Construction*

A dictum of this brain modelling course is: "one must be able to defend any structure which one adds to the model with all the

evidence to be found." This calls for a thorough acquaintance with the information contained in the standard neuroanatomy textbooks, the current literature, and the gross and microscopic materials at hand.

The color scheme for the model is as follows:

Motor nuclei, peripheral nerves. Pyramidal tract.....Red
Caudate, Putamen .....Terra Cotta
Globus pallidus and connections.....Black
Associate Plate
(reticular substance, intra and intersegmental connections).....Gray
First Afferent Neurons
Sensory nerves, ganglia (Receptor plate), mesencephalic and spinal roots of 5th, tractus gracilis and cuneatus.....Light blue
Second Afferent Neurons
Superior sensory and spinal nuclei of 5th, secondary 5th tracts, nucleus gracilis and cuneatus, medial lemniscus, substantia gelatinosa Rolandi.
Spinothalamic tract .....Dark blue
Third Afferent Neurons
Nucleus ventralis posterior.....Light green
Postcentral cortex .....Dark green
Vestibular nucleus and connections (including flocculo-nodular lobe) .....Purple or Violet
Other Cerebellar Connections
Afferent:
Paleo-cerebellar .....Light yellow
Neocerebellar .....Dark yellow or Orange
Efferent:
Paleocerebellar .....Light brown
Neocerebellar ..... Dark brown
Medial longitudinal fasciculus, vestibular spinal tract, olivo spinal tract ..... Black
Auditory system, including medial geniculate body and auditory radiations .....Dark green
Optic nerve and tract.....Light blue
Lateral geniculate body and optic radiations.....Dark blue

Structures not listed are made in any color desired, but in general, *red* indicates motor and *blue* indicates sensory.

The model is to be constructed 4 times the size of an average adult brain. Unless the structures of the model are built in a proper sequence, architectural difficulty is soon encountered. The procedure described below has been established from the profits of many mistakes and lost hours:

1. Assemble all of the wooden parts with bolts and screws as shown in figure IV, but do not glue the rods in place. It is found

that the carpentry is much easier if all the parts are drilled and secured at the beginning of the course. The parts can then be removed as desired for better access during the process of modelling.

2. Paint the baseboard with two coats of Spar varnish, so that drawings can be made with India ink without spreading into the porous wood, and errors can be erased by scraping.

3. Drawings of cross sections of the spinal cord in the cervical, thoracic, lumbar, and sacral regions, using India ink for the outlines and paint for the tracts according to the color scheme, are then made on the baseboard. Instead of small brushes which necessitate constant cleaning, it is advisable to substitute wooden swab sticks or toothpicks, beveled to a point, in painting the many colors of the baseboard. The wooden sticks are almost as good as artist brushes, and much more economical.

4. A drawing of the various types of peripheral nerve endings (Fig. VI) is now made and connected diagrammatically with a cross section of the spinal cord on the small, elevated block of wood, into which is fitted the cylindrical rod supporting the brain stem structures. According to the scale of 4 x normal, the cross section of the spinal cord on the small block of wood will be at about C4 level. Besides the various types of peripheral nerve endings the special sense organs, such as the retina, cochlear, semicircular canals, etc., may also be drawn on the baseboard, as shown in figure VIII.

5. After all of the drawings are completed on the baseboard, a third coat of Spar varnish is applied and, when dry, the baseboard is covered with wrapping paper to prevent damage during subsequent modelling.

6. Using grey plasticine, the reticular formation is molded around the supporting rod. In order to prevent the plasticine from falling off, small holes are drilled through the supporting rod, and wires are inserted and woven to form a rough framework of the reticular formation prior to the molding (Fig. I).

7. Motor nuclei and medial longitudinal fasciculus: The wooden framework in the midline may interfere with the construction of the different components of the oculomotor complex which, consequently, can be represented only diagrammatically. The lateral nuclei of the oculomotor nerve are visible on either side of the rod, and the nucleus of Perlia is hidden, while only a

portion of the Edinger-Westphal is seen from the surface. The placement of the trochlear, abducens, and hypoglossal nerve nuclei involves no problem (Fig. I).

The motor nucleus of the trigeminal nerve and the facial nucleus are imbedded in the plasticine reticular substance. The dorsal motor nucleus is a complex unit, which consists of a vagal component and a glossopharyngeal component at its cephalic portion. The separation of these two components in the dorsal motor nucleus cannot be made on morphological grounds. The spinal accessory nerve nucleus is a group of motor cells in the anterior substance of the reticular formation, extending from the level of the pyramidal decussation to that of the first cervical segment.

The medial longitudinal fasciculi are represented by a pair of black strips of plasticine along the dorsal medial surface of the reticular substance. Their oral extension reaches the transitional zone between the hypothalamus and mesencephalon. They disappear caudally from the dorsal surface of the obex, where they penetrate the reticular substance and emerge from its ventral surface. The ventral portion of the medial longitudinal fasciculi is represented by black radio wires, which course downward into the marginal fasciculus of the spinal cord section on the elevated baseboard.

8. Receptor plate and receptor nuclei. Dark blue plasticine is used for the sensory nucleus of the trigeminal nerve. Oral extension of this nucleus with light blue plasticine forms the mesencephalic root. It is necessary to mold the mesencephalic root over a piece of wire for support. The spinal tract descends and fuses with the tract of Lissauer in the cross-section of the cervical cord at the base.

The nucleus solitarius is made with blue plasticine, inside which is a blue wire, representing the tractus solitarius.

9. Gracile and cuneate nuclei, and medial lemniscus. The two large sensory nuclei are molded with blue plasticine. They are connected caudally with the gracile and cuneate tracts by blue radio wires. In addition, the lateral cuneate nucleus can be placed.

At this stage, the small wooden piece representing the cross-section of the right posterior thalamus is ready to be bolted in

place at the level of the red nucleus. On the anterior and posterior surfaces are outlined the nucleus ventralis postero-lateralis and nucleus ventralis postero-medialis. Several small holes are drilled in the lower part of these nuclear areas, so that wires can be secured as fiber connections are added later. This small block of wood (Fig. IV) is attached to the framework at a slight angle to the supporting rod shown in figure V. It is not visible in figure VII because the basal ganglia hide it from view at a later stage.

The large medial lemniscus is then molded with blue plasticine around several wires which are fastened above to the nucleus ventralis postero-lateralis. The internal arcuate fibers are designated by small blue wires from the gracile and cuneate nuclei to the opposite thalamus.

10. Cerebellum, vestibular nuclei, and connections. The cerebellar plate (sagittal section) is painted diagrammatically to show the various lobes. At the proper position on the plate, a metal rod is inserted, to bear up the dentate and roof nuclei at a later stage.

The vestibular nuclei are then added by using purple or violet plasticine. The connections of the vestibular subdivisions with the medial longitudinal fasciculi are installed with small strips of black plasticine.

The dentate, emboliform, and globose nuclei are molded and placed. So are the fastigial nuclei and flocculo-nodular lobes. The latter structures and the vestibular system are connected by small radio wires.

The brachium conjunctivum is a heavy structure which can be easily secured, when the dentate and the red nuclei are firmly held by screws and wire frames. The rubrospinal tract is small and is marked by a red wire.

The right inferior olive is made with a galvanized wire mesh basket, anchored to the wood and covered with yellow plasticine. Its connections with the cerebellum are represented by yellow radio wires passing through the medial lemniscus. The dorsal spinocerebellar tract is now added with yellow wire and plasticine, and joined to the olivo-cerebellar fibers to form the major part of the restiform body. With similar colored wire and plasticine, the ventral spinocerebellar tract is represented as it courses upward and backward to the cerebellum via the brachium conjunctivum.

11. Spinothalamic tracts and secondary trigeminal connections. At this stage the sensory pathways are to be completed. These fibers are represented by blue wires, which terminate at the nucleus ventralis postero-lateralis and nucleus ventralis postero-medialis respectively.

12. Cochlear nuclei, trapezoid body, lateral lemniscus, superior olive, and inferior colliculi. These structures which form the auditory system are all green on the model. The cochlear nuclei, dorsal and ventral, are placed only on the left side of the model and the connecting fibers are carried over to the trapezoid body, superior olive, nucleus of lateral lemniscus, and the contralateral lateral lemniscus. The latter structure ascends and terminates at the inferior colliculus. The auditory radiations and medial geniculate bodies are not constructed until later.

13. Hypothalamus. With different colored plasticine the various hypthalamic nuclei are distributed on the lateral wall of the third ventricle according to LeGros Clark (1936). The hypothalamic connections are not constructed until later.

14. Thalamus. On the left side of the model, a wire basket, slightly smaller than the calculated size of an average adult thalamus, is bolted to the midline wooden plate at the proper position. The basket is then covered with a shell of plasticine in various colors, denoting the different thalamic nuclei on the surface of the thalamus. On the right side, four representative cross-sections, anterior to the level of the mammillary body, are drawn on the front and back surfaces of two wooden pieces which are attached to the midline wooden plate. The various thalamic nuclei are distinguished by different colored paints. The construction of the thalamic connections is postponed until later.

15. Striatum and pallidum. Building the putamen and globus pallidus as a unit, the anterior end of which merges with the head of the caudate nucleus, is accomplished with the use of wire baskets which are firmly attached to the right coronal wooden hemispheric section. The globus pallidus is then indicated by molding a shell of black plasticine on the medial portion of the basket, and terra cotta plasticine on the lateral portion for the putamen. The latter is connected with the caudate nucleus. The body of the caudate runs backward and the tail downward and forward, finally fusing with the amygdaloid.

16. Base of skull and cranial nerves. The left anterior, middle

and posterior fossae are represented by a wire mesh framework with openings corresponding to all foramina of the cranium on one side. In addition, the petrous ridge is built. These are coated with white plasticine. The cochlea and semicircular canals are placed. All the cranial nerves are represented by wire and plasticine from the nuclei to their respective foramina (Fig. VII).

17. Optic nerves, chiasm and tracts, lateral geniculate bodies, optic radiations, medial geniculate bodies, and auditory radiations are now added. The optic system is constructed with light blue and dark blue plasticine and wire, while the auditory system is constructed with dark green plasticine.

18. Olfactory system, amygdaloid, and anterior commissure. The olfactory system is built with yellow wire and plasticine, and consists of the olfactory nerves, bulbs, tracts, medial and lateral olfactory stria, stria longitudinalis, stria semicularis, diagonal band of Broca, and the fibers in the anterior commissure. Green, yellow and black plasticine are used for the construction of the amygdaloid complex. When the above structures are all in place, the hippocampus, fimbria, dentate gyrus, fornix, mammillothalamic tract, habenular commissure, stria medullaris, and the indusium griseum over the corpus callosum are added.

19. Choroid plexus and cortex. The choroid plexus is represented by interweaving red wool yarn about a wire. The precentral and postcentral cortical areas for the leg, arm, and face are denoted by pieces of wire mesh on the convexity of the hemispheric plate. Areas other than those mentioned are labelled on the plate according to their cortical representations. The calcarine cortex is constructed with plasticine of various colors according to the orientation of the retina and the geniculo-calcarine fibers. The auditory cortex is added to the hemispheric plate over an area corresponding to the transverse convolution, which is connected to the medial geniculate body by radio wires.

20. Substantia nigra, subthalamic nuclei, Forel's fields are added.

21. Completion of all fiber connections.

22. Labelling and varnishing.

Three views of the finished model are shown in figures VIII, IX and X. No two models ever look exactly alike because some workers seek to make the model very realistic, and others use it to show the structures and their relationships in the simplest

diagrammatic form possible. Some add connections which are based on physiological experimental evidence, and others add only the gross anatomical structures or structures proved by anatomical methods only. Both groups are encouraged to add what they wish, as long as they can defend what they add. Controversies arising from disputed connections have led some workers to perform experiments of their own, to confirm or deny the existence of these connections.

IV. *Discussion*

Brain modelling is a flexible operation, and additions to the model will undoubtedly be made as knowledge of neuroanatomy advances. The earlier ideas in this course, that the reconstruction of the anatomical units in the model should be based purely upon embryological, anatomical, and comparative neurological findings as advocated by Adolph Meyer, have been modified. For the sake of discussion and argument, certain structures, the existence of which has been substantiated only by physiological experiments, have also been included in the model. This applies especially to the fiber connections.

For teaching purposes, this course is best suited to students with a background in neuroanatomy, who will obtain a three dimensional impression of the structures through molding them by hand. It should be kept in mind that the initial function of brain modelling is in its being a method of studying neuroanatomy, rather than a challenge to the most realistic and elaborate representation of the brain.

The enthusiasm, with which the students start out, frequently wanes when their ambitious schemes of construction prove difficult or unworkable. To sustain interest, it is wise to employ a schedule which consists of weekly seminars on problems, corresponding to the stages of development in the modelling. The seminars are conducted by students in rotation, with one hour of prepared talk on a particular subject followed by one hour of discussion. Twenty such seminars take place throughout the course, and during the final seminar, the models (theoretically in completion) are lined up for mutual inspection and criticism.

The advanced scholars, to whom joining a brain modelling course would be impracticable or superfluous, may find the diagrams and suggestions given here helpful in their individual

study, whether it is a first venture into the field or a review of earlier work.

V. *Summary*

1. A brain modelling course for the study of neuroanatomy is described.

2. The emphasis is laid upon an understanding of the subject rather than the architectural procedure.

3. The technical suggestions given are applicable to class instruction as well as individual study.

Acknowledgment:

The authors wish to express their gratitude to Dr. Francis McNaughton for his guidance in the study of neuroanatomy at the Montreal Neurological Institute.

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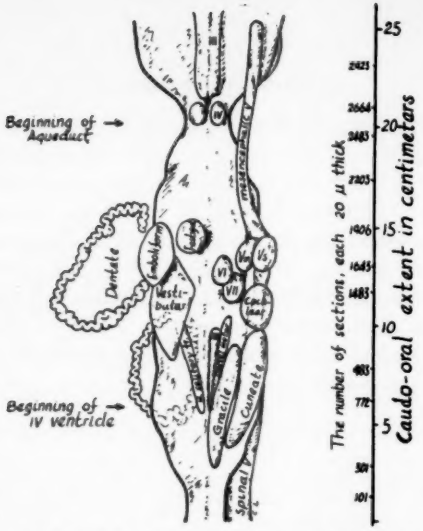
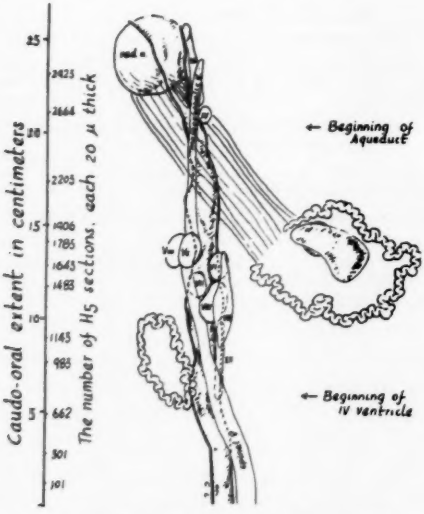


THE DORSAL VIEW OF RETICULAR FORMATION AND RELATIVE SIZE, SHAPE & POSITION OF THE NUCLEAR MASSES IN BRAIN STEM. x 4

FIG. I. Dorsal aspect of the nuclear masses and of the reticular formation of adult human brain stem. Scale times 4. The number of sections corresponds to that of the Nissl and Heidenhain series, H5, provided by Dr. Jerzy Olszewski.



THE LATERAL VIEW OF THE MIDBRAIN RETICULAR FORMATION AND CRANIAL NERVE NUCLEI, ADULT HUMAN. x 4

FIG. II. Lateral aspect of the nuclear masses showing their relative size, shape, and position in the reticular formation. The dentate, emboliform, and fastigial nuclei of the cerebellum and the inferior olive are also shown. Scale times 4.

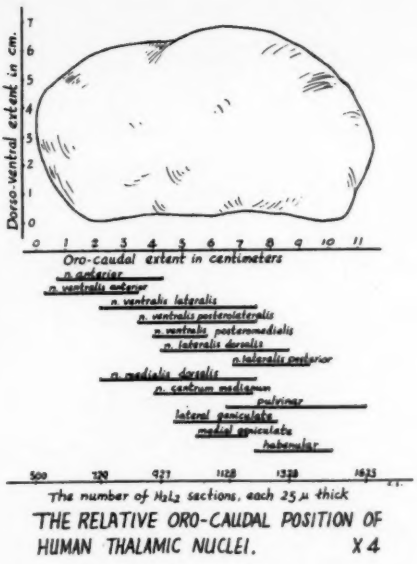

Dorso-ventral extent in cm.
0 1 2 3 4 5 6 7 8 9 10 11
Oro-caudal extent in centimeters
n. anterior
n. ventralis anterior
n. ventralis lateralis
n. ventralis posterolateralis
n. ventralis posteromedialis
n. lateralis dorsalis
n. lateralis posterior
n. medialis dorsalis
n. centrum medianum
pulvinar
lateral geniculate
medial geniculate
habenular
500 729 927 1128 1328 1525
The number of H&L2 sections, each 25 μ thick
THE RELATIVE ORO-CAUDAL POSITION OF HUMAN THALAMIC NUCLEI. X 4

FIG. III. The relative position and oro-caudal measurements of the essential nuclei in an adult human thalamus. Scale times 4.

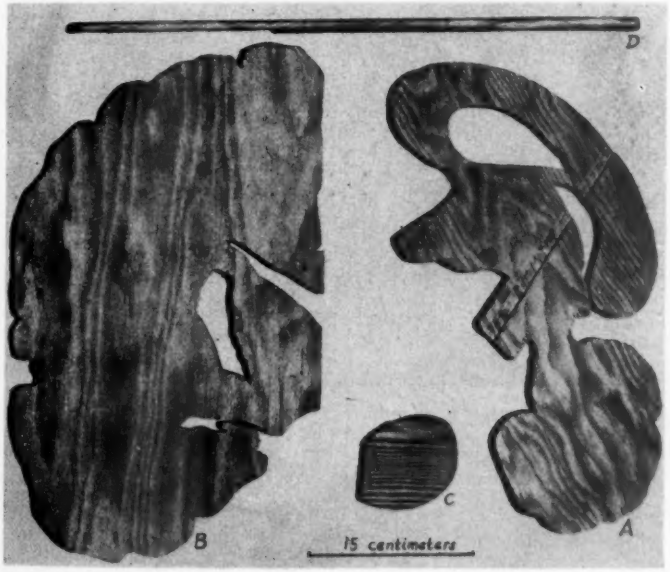


FIG. IV. Components of wooden superstructure cut to scale of four times the actual size. A. corpus callosum and cerebellum. B. cross-section of the right cerebral hemisphere. C. cross-section of the right thalamus at the level of the red nucleus. D. cylindrical rod.

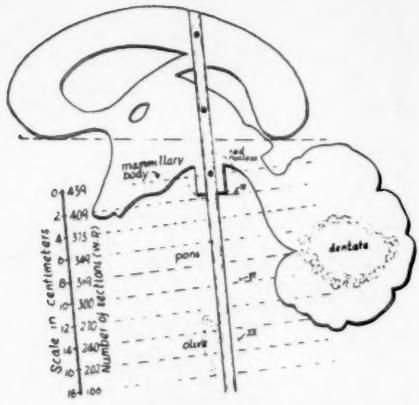


FIG. V. The wooden framework of a brain model. Note that the mammillary body and the red nucleus are at about the same level.

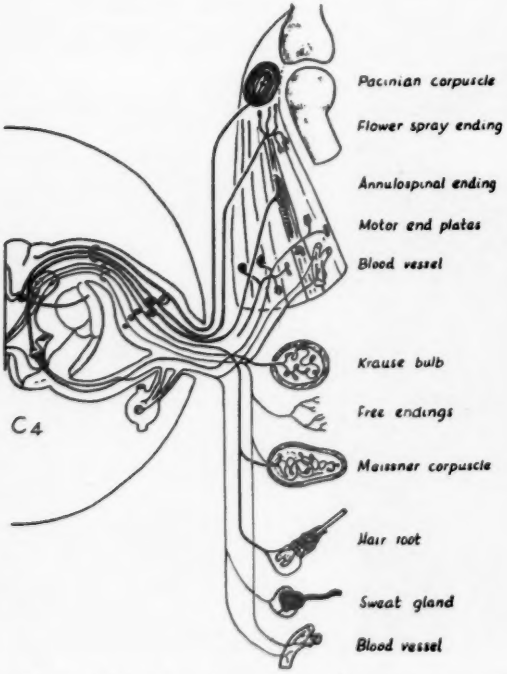


FIG. VI. On the baseboard of the brain model is a diagram showing the major afferent and efferent nerve endings and the specialized receptive organs.

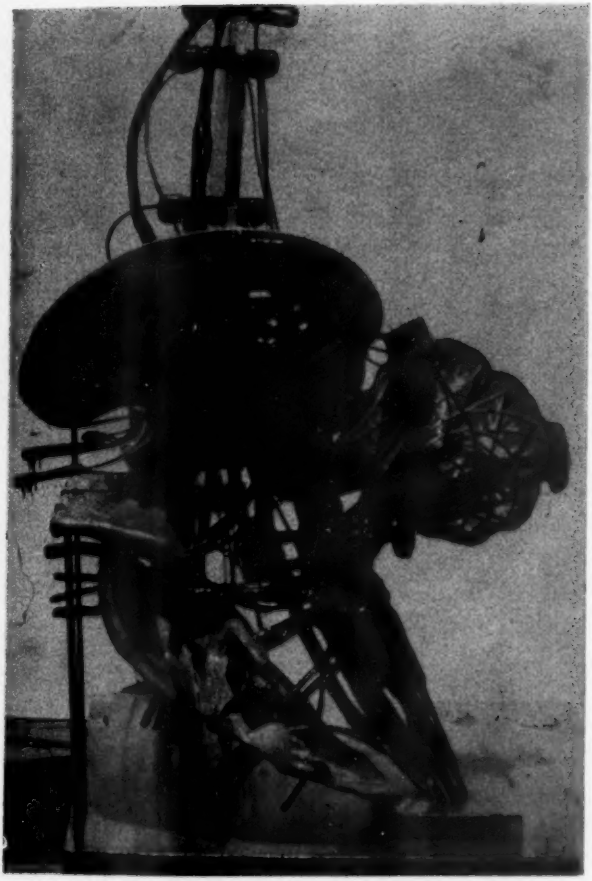


FIG. VII. A brain model showing the base of skull and the exits of cranial nerves.

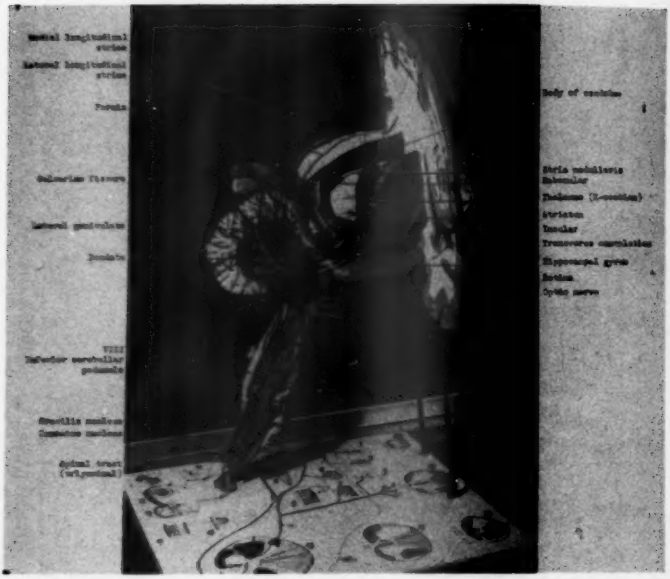


FIG. VIII. Lateral posterior view of a brain model (constructed by Mr. Charles Wood of the National Institute of Neurological Diseases and Blindness). In this and subsequent illustrations only the essential structures are labelled. 1. Meissner's corpuscle, 2. Pacinian corpuscle, 3. Basket endings, 4. Merkel's disc, 5. Ruffini's corpuscle, 6. Krause end bulb, 7. Free endings, 8. Muscle spindles, 9. Motor end plate, 10. Cross-section of cochlear showing nerve endings in the tectorial membrane. 11. Cross-section of one turn of cochlear showing the Organ of Corti and spinal ganglion, 12. Cochlear, 13. Semicircular canals, 14. Crista of semicircular canals, 15. Macula of retina, 16. Cross-section of first cervical segment, 17. Seventh cervical segment. 18. Eighth thoracic segment, 19. Third lumbar segment, 20. Fourth sacral segment.

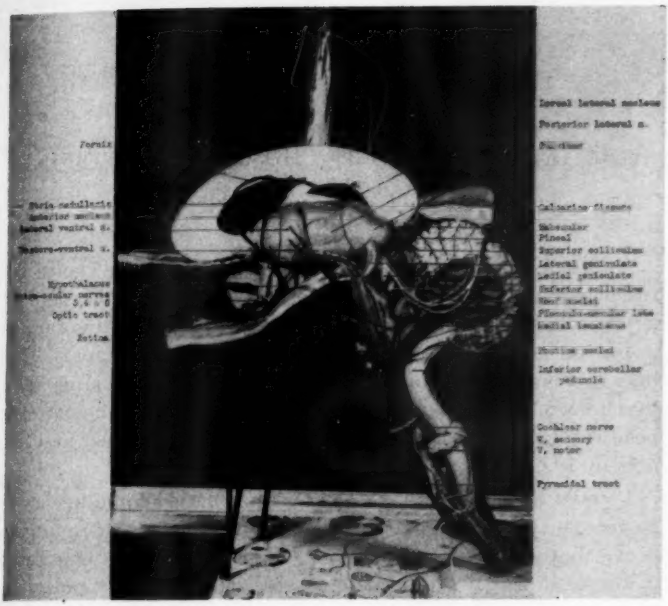


FIG. IX. Lateral view of a brain model.

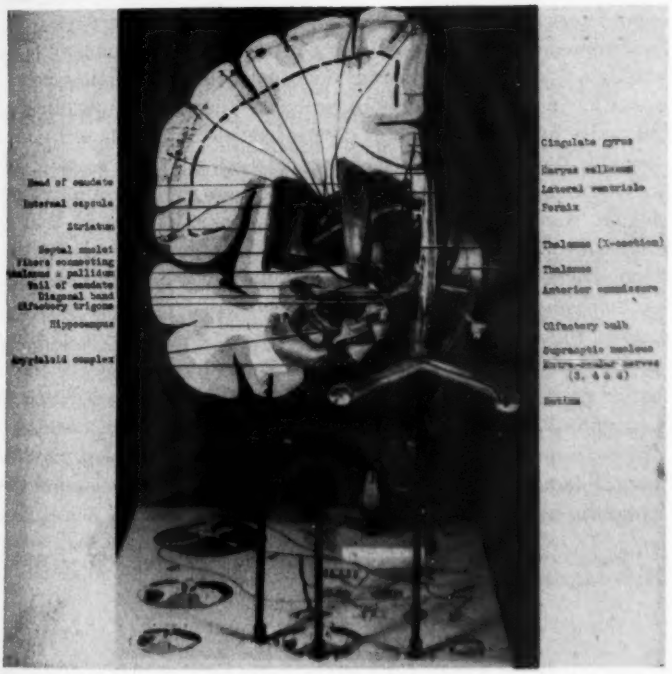


FIG. X. Anterior view of a brain model.

SOME DILEMMAS OF PSYCHOSOMATIC MEDICINE

MARCEL PATTERSON*

A dilemma is defined as a situation involving the choice between equally unsatisfactory alternatives. The whole field of psychosomatic medicine is filled with dilemmas. I will not try to prove that certain diseases arise from psychological causes. This may or may not be true. At the moment proof is lacking. That psychological disturbances can cause visceral malfunction has been recognized for centuries and was well established by Cannon in 1929.

I would like to reiterate from Ziskind (1954) some factors to be recognized when one studies or reads articles attempting to prove that an organic condition is of psychic origin. One must distinguish primary or specific causes from secondary causes. A disease to be truly psychosomatic should be a condition that could not occur in the absence of specific psychological factors and would not clear up unless the psychological factors were removed or disappeared. The presence of contributory or secondary psychological factors in a disorder does not justify calling it psychosomatic since such factors are probably operative in all disease. Secondly, in diseases such as ulcerative colitis, asthma or hypertension, the finding of psychological factors, alike in cases of known and unknown origin, cast doubt upon their specificity and suggest that these factors are secondary. The first group, that is those of known origin, might serve as excellent controls for the second group, but in most studies this has not been done. Thirdly, predisposing causes should be separated from precipitating causes. Precipitating causes are usually not primary nor specific, particularly in such paroxysmal or remittant diseases as those called psychosomatic. For example, the exacerbation of visceral lesions under stress in an individual who has one of these conditions is not proof of stress etiology. Fourthly, different psychological factors have been said to be the specific causes for the same disease. Fifthly, proof of psychological causation of func-

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tional visceral changes should not be accepted as evidence of psychological origin of demonstrable organic changes.

Let us discuss some phases of the subject of visceral malfunctioning due to psychological stress. The occurrence of this phenomenon is almost a universal observation in each of us, and is well documented. In this paper three phases of this subject will be explored. The first is the origin of psychological stresses. emotions, fears, or conflicts. Secondly, the site and pathways by which these disturbances are transmitted and thirdly, a consideration of the end organs reaction.

Psychic disturbances are either *internally-derived* (that is, arise within the individual's conscious or unconscious mind, *the I*) or *externally derived* (the reaction of the environment on the individual, so-called stressful life situations, the *NOT I*). What is operative in most instances is the reaction or combination of these two factors. It is recognized that all inner-derived conflicts must have derived originally from the outside world. Spontaneous instinctual drives frequently clash with learned standards of behavior and thought. For our discussion we are thinking in terms of symptoms (visceral malfunctions) as they arise in a unique individual in a particular place at a specific time, and not in the abstract.

Inner-derived conflicts are usually considered the province of the psychiatrist. Here we are confronted with considerable confusion as to the more important factor or factors in the origin of these conflicts. As Ziskind (1954) points out, psychiatrists and psychiatric literature follow three large schools; the psychoanalytical school, the psychobiological school, and the eclectic school. The psychoanalytic school, like Christian theology, has broken up into several different camps. Each of these schools conflict to some extent in what they consider man's more basic drives and adjustments. All agree that frustration of these drives is the origin of conflicts. Most psychiatrists assume that once the origin of the conflict is understood and emotionally relived the symptoms disappear. Each view is highly systematized and has developed an extensive literature and language of its own. This is particularly important to recognize if one hopes to read an article and come away with any meaning. One should know, if possible, what school the writer follows. This usually can only be deduced provided one is aware of the symbolic language that goes with

each school. The vast majority of writings on psychosomatic medicine come from analytically oriented psychiatrists. Much of it is highly theoretical. Since it requires no traditional training in anatomy, physiology, or the grass roots of medicine, it has been assimilated by the public in tremendous quantities and has been accepted as if it had met Koch's postulates for disease.

I am not attempting to debunk all psychoanalytically oriented explanations of disease. This point of view has made me recognize that conflicts may be so deeply buried that they disturb and plague us without our being able to scratch the so-called itch. In handling patients I assume, when I do not have a concrete stressful situation to explain symptoms, that either the patient is unwilling or unable to confide in me or that the conflicts are such that he is unable to recognize them. In either situation what I have to offer this patient will not be of much help in solving his difficulties. And, secondly, these factors are operative in all of us to some extent. Given the proper situations it seems extremely likely that any one of us might develop neurotic patterns of behavior. Consequently, one can approach a patient without the depreciative attitude that usually goes with the label "neurotic" or "functional illness."

The psychobiological school of psychiatry is considered to be the only truly American school of psychiatry. It was founded by Adolph Meyer at Johns Hopkins. It has the healthy attitude of a pluralistic explanation for behavior and it helped tear down the 19th century concept of duality of mind and body. It conceived of mind as another organ system in the total body physiology characterized by the unique capacity of symbolization. An aberration, whether it manifests itself primarily as psychotic behavior or somatic dysfunction, is a reaction to a noxious experience. There is an insistence on linking structure and function. The greatest handicap to this school was in Dr. Meyer's usage of strange labels which never displaced the terms already fixed in psychiatry. Some of the contributions were: the salubrious effect of stressing to the physician the consideration of the patient as a whole, total man: the emphasis upon multiple etiological factors; individualization of each case; a centering of attention upon the complaint and a common sense evaluation of life situations. It also gave encouragement and comfort to those individuals who

felt that psychic phenomenon might some day be measured and observed with the same scientific objectivity that had been applied to other body physiology.

The eclectic school includes the great bulk of psychiatrists who feel that the knowledge of mind and emotions is as yet inadequate to warrant a formal system; that factual knowledge is extremely fragmentary and before one accepts any explanation of behavior more objective scientific data are needed. There is an obvious need here for all of us and particularly those who are concerned with research to define methodology in this particular field.

The external factors which give rise to psychic disturbances make up a large group of heterogeneous subjects. These include such things as interpersonal relationships, familial relationships, occupational situations, race, nationality, folkways, mores, religious beliefs, political beliefs, and a great variety of seemingly unrelated subjects that include such fields as sociology, theology, anthropology, climatology, and even geography. We are all aware from our personal experience that conflicts in these spheres may cause somatic symptoms. Since many of these factors can be adjusted more readily than deeply ingrained behavior patterns much of short term psychotherapy is involved in helping the individual either to modify, withdraw or accept these external influences. These factors are more readily observable and measurable and should be measured more often for clearer understanding of somatic reactions to environmental stress. Unfortunately little or no time is spent in the medical school curricula on cultural and environmental factors as related to behavior and disease, that is, in regard to the individual, not overlooking the great amount of material that is offered in preventive medicine and public health in the control of society's physical environment. The physician becomes aware of the tremendous impact of these factors when he is confronted with the care of a patient in his practice and recognizes that many of his well learned diagnostic procedures and therapeutic measures cannot be carried out because of environmental or cultural factors.

This brief resumé of some of the origins of conflicts emphasize that the problem is a tremendous one. Any one simple or rigid

formula for behavior is probably inadequate. Until more objective methods for measuring psychic phenomena are evolved and until more observations are made of those factors that are measurable, the field will probably continue to be more metaphysical and philosophical than scientific. Fortunately there need not necessarily be any close correlation between successful therapy and theory. The argument that the theory is correct because the therapy is successful, is fallacious. By the same argument, the theories of Aimee Semple McPherson, the Palmer Method of Chiropractic and the hysterical incantations of the African witch doctor against evil spirits are all valid. And finally, I strongly suspect that in many instances the patient is not so much relieved of his symptoms as, in accepting the explanation, he learns to tolerate them without the added anxieties and fears of the unknown and thus does not incessantly demand to be cured.

Consider a second phase of this problem. That is, the location of the site of conflicts within the anatomy of the brain and their pathways to various organs. This problem has vexed learned minds for years. To explore it properly would necessitate a study of the fascinating subject of the location of mind and memory, because an emotion, whatever its origin must have a relationship to past experience (memory), and future action. The problem is truly so complex that if one gives the matter serious thought it seems to be almost staggering.

For our purposes we can say that emotion as experienced by man is a cortical function. As Gillilan (1954) emphasizes, it is not eliminated by cutting the vagus nerve or sympathetic chain, nor by blocking visceral, skin or muscle sensation. The seat of this cortical activity is probably located in the temporal and frontal lobes.

Magnus et al. (1952) have shown that stimulation of the lower temporal lobe in man may produce anxiety and also certain vague epigastric and precordial symptoms that resemble spontaneous anxiety. Similar observations have been made by Gibbs (1951), Mueder and Daly (1952), and Penfield (1947) on patients with epileptic seizures with foci in these areas and in patients with severe trauma to these areas.

Heath (1954) notes that the basic change in psychosurgery, particularly prefrontal lobotomy, is a lessening of concern for

the future. Von Bonin (1952) reports that the most obvious differences between the brain of man and monkey is the extensive development of the frontal and temporal cortex. The principal behavorial characteristic accompanying this anatomical development is the ability to anticipate the future in terms of past events. Much of the symptomatology of "functional" illness is not characterized by severity, that is in terms of objective observation, but is primarily the interpretation by the patient of the significance of the symptoms in relation to his future integrity. Penfield (1952) proposes that there is a neuron system centrally placed within the brain equally connected with the two hemispheres. This system is situated somewhere in the brain stem (including the thalamus, subthalmus and hypothalamus). The location is uncertain. It is provided with wide spread connections to and from the cortex of both hemispheres. This he defines as the "centrencephalic system." The indispensable substratum of consciousness or awareness lies outside the cerebral cortex, largely in the diencephalon. Memory mechanism, the records of an individual's thinking, lie dormant in the patterns of his temporal cortex until he activates them in some normal process of recall or until they come into spontaneous and perhaps distorted existence, as for example in dreams.

One gets the impression that the processes of memory and mind and of necessity the processes of emotion are stored somewhat like library books in the temporal and frontal lobe while co-ordination of these processes is in the "tween" brain or "centrencephalic system."

Some people have postulated that the hypothalamus is a coordinator or a regulator of these various mental processes. External stimuli, by evoking a prior experience in memory, or internal stimuli (Penfield postulates the origins of these in the centrencephalic system) set into action a series of (and I use this word for lack of a better one) synapses. Spiegal in 1928 noted close topographical relationships between cortical areas from which viscera can be activated and those which excite adjacent somatic structures. Gillilan (1954) in a recent book correlates a great deal of experimental work and maps certain cortical areas of practically all visceral function. She concludes that automatic function is represented throughout the cerebal

cortex in a close spatial relationship to the representatives of adjacent somatic structure. Some people have felt that the location of this function in the cortex was in the reticular system and others have applied the term visceral brain. She admits that these areas are not as well defined as external somatic structures, but she sees this cortical activity as primarily an inhibiting mechanism over the hypothalamus which in turn regulates the lower brain stem and cord centers. Removal of cortical influences results in a release of the hypothalamus and in gross autonomic disturbances. Perhaps there are instances where distortion of cortical influences rather than removal result in the distortion of the co-ordinating mechanisms of the hypothalamus and gross autonomic disturbances.

For years one of the proofs of relationship between brain and visceral abnormalities was in the observation of acute ulcerative lesions of the gastrointestinal tract associated with organic brain damage. Recently Brick and Jeghers (1955) reviewed a great deal of experimental evidence and articles that have been reported on this subject. Whether these lesions are the result of actual removal of cortical influences on the autonomic nervous system with a resulting imbalance or whether they are the result of the general stress pattern as proported by Selye are argumentative points. For example, French and co-workers (1954) noted ulcerations of the acute type in cats and monkeys following stimulation of the hypothalamus by electrodes; while Davis and co-workers (1955) found no lesions in cats after destructive lesions in the hypothalamus. They point out that these ulcerations have occurred following laminectomy, hemilaminectomy and trigeminal neurectomy and believe that the individual somatic lesions probably arise through pituitary hormonal stimulation of the adrenal gland. At any rate, the pathways of transmission must be either hormonal or neurogenic and perhaps in many instances both factors are operative. Whether the hormonal effects arise from the direct action of the hypothalamus on the pituitary through nerve pathways (known to exist), through neurosecretion, or whether they come from the release of epinephrine, is a controversial point. It would seem reasonable to believe that in any severe stress situation all pathways might be operative. Altschule (1954) has summarized the evidence

which would indicate that epinephrine or some of its derivatives cause feelings of anxiety through a central action that is independent of the pressor effect. Thus, one has cyclic mechanisms for maintaining stress patterns once the mechanism is put into action.

The third phase of the problem that I would like to discuss is the organ reaction, not the reaction itself which has been well documented by various writers particularly Wolf and Wolff (1947 and 1951) and need not be restated at this time, but rather why in one individual certain organ physiology should be disturbed out of all proportion to another organ system. The pattern is rarely that of total stimulation by sympathetics or parasympathetics. Psychoanalytically oriented workers postulate a specific organ reaction as a counterpart of a specific emotional crisis. For example, Alexander (1950, Pg. 47) states "just as certain pathological micro-organisms have a certain specific affinity for certain organs, so also certain emotional conflicts possess specificities and accordingly tend to affect certain organs. Inhibited rage seems to have a specific relationship to the cardio-vascular system; dependent help-seeking tendencies seem to have a specific relationship to the functions of nutrition. Again, a conflict between sexual wishes and dependent tendencies seems to have a specific influence upon respiratory functions." He further classifies cultural disturbances into two categories: those which lead to withdrawal from all external problems and retirement into a more vegetative existence (the ulcer, colitis and the asthmatic patient) and the attitude of turning to external environment with preparation for flight or fight (the hypertensive, the rheumatoid arthritic and thyrotoxicosis patient). Most controlled experimental work indicates that a variety of stress situations can cause similar reactions in various organs. And while the specific organ theory is interesting it lacks proof.

Another explanation of individual patterns of autonomic response to emotion is the conditioned reflex. Brunswick in 1925 showed that gastro-intestinal motor patterns varied with emotions but were consistent in individuals and subjects studied.

Lacey (1950, 1951 1952, 1953) and Terry (1953) using simple stress situations obtained autonomic responses which varied from individual to individual but these responses were consistent

in the same individual. This would tend to indicate that the autonomic nervous system does not respond as a whole but that there is an autonomic response specificity. A recent study by Jorup (1952) in infants suggests that there is an inherited reaction pattern; Bindra and Thompson (1953) in a study on rats reached similar conclusions. These studies of course do not exclude patterns acquired in early life. But these explanations seem better than Cannon's theory of either fight or flight reaction with predominately vagotonic or sympathetic tone. The conditioned reflex theory is attractive and as Altschule has pointed out is supported by the following findings:

1. Studies on neurotic or unstable subjects reveal that the establishment of reflexes is accelerated.
2. Condition responses occurred when neurotics were given stimuli too different from the conditioning stimuli to evoke a response in normal individuals.
3. The conditioned reflex is stronger on retesting after longer, rather than shorter periods of rest; this would indicate that the reaction associated with anxiety and emotional stress may incubate.
4. Voluntary and conscious mental attitudes do not affect the phenomenon.
5. Merely thinking about the stimuli may evoke the response.
6. Conditioned reflexes have been observed after simuli that are not consciously perceived.
7. The number of stimuli to which one may come to respond is extensive and the individual may be made to respond in many ways, possibly infinite. A systematic study of response to standard stimuli might be revealing.

In this review I have attempted to take a long range view at some of the problems or dilemmas involved in the understanding of psychosomatic medicine. There are glaring omissions, particularly in the field of pharmacology and the reactions themselves which most books on the subject enumerate in detail.

Possibly some functional illness may be associated with metabolic defects which have not yet been discovered. Stevenson (1955) summarizes reports which would indicate that this may be the situation in some cases of schizophrenia and of mental retardation. If this paper stimulates an interest in this field the time has been well spent. The importance of psychosomatics in medical practice cannot be overestimated.

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THERMAL STRESS AND TISSUE CULTURE*

VII. The Effect of Glycerol on Cells with Special Reference to Protection Against Freezing

C. M. Pomerat and P. S. Moorhead†

The introduction of glycerol in the medium used for the freezing of spermatozoa by the investigators at the National Institute for Medical Research in London has led to extraordinarily successful methods for the storage of animal cells. The practical significance for animal breeding of long term preservation of sperm has been fully established. The goal of similar banking of erythrocytes for use in transfusions already has shown great promise (Brown and Hardin, 1953). Another purpose which has been achieved is the long term storage of valuable pure cell culture strains. Scherer and Hoogasian (1954) have shown that Earle's Strain L frozen in 5 per cent glycerol and Gey's HeLa cell frozen in 20 per cent or 30 per cent glycerol can resist a rapid drop in temperature to —60° or —70° C. with thawing in 1-2 minutes. Viability for these elements after more than one year of storage at low temperature has been established (Scherer —personal communication—1955). The freezing process is valuable in the field of cell culture in assuring reliable reference biological materials and for many aspects of the practical management of test objects, particularly for studies in virology and oncology. From the surgical point of view, long term preservation of tissues for purposes of grafting procedures has led to the freezing of various organs, particularly those belonging to the endocrine system (*cf.*, Smith, 1954).

The purpose of this investigation was to study the effect of glycerol on cells with the use of techniques which have been developed in the course of assembling data for a series of papers under the general title: *Thermal Stress and Tissue Culture.* Three lines of attack were undertaken:

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A. A comparison of the value of glycerol as a protective agent in the freezing and thawing of tissue materials regarding which considerable information has been accumulated. Primary explants rather than cell strains were employed in the hope that information could be obtained on the interrelations of parenchymatous versus stromal elements following thermal stress (*cf.*, Nakai, Pomerat, and Gilliland, 1954). Previous work with cell cultures frequently has revealed that factors enhancing the outgrowth of fibroblasts retard the emigration of epithelium from skin and of multinuclear fibers from muscle explants.

B. The effect of glycerol on cell organoids. In addition to the protective action in the course of freezing and thawing operations, the relatively non-toxic action of glycerol at high concentrations which induces marked changes in the physico-chemical properties of cells poses a fundamental cytological problem. With the use of phase photomicrography, it was proposed to make records of some of the changes in cell organs induced by glycerol.

C. The effect of glycerol on the activity of cells as recorded by phase contrast, time-lapse cinematography. A perfusion chamber technique currently employed in this laboratory for the analysis of cell reactions to environmental changes was well adapted for such study (*cf.*, Pomerat, Lefeber, and Smith, 1954).

A. *The Effect of Glycerol on the Freezing and Thawing of Chick Tissues*

The materials and methods employed for recording the freezing and thawing of chick embryonic tissues were identical with those described in the first publication in this series (Pomerat and Lewis, 1953 a, b). Since Gey's balanced salt solution (BSS) had been found to be a satisfactory medium for the rapid freezing and thawing of tissues (Nakai, Pomerat, and Gilliland, 1954), this was employed as a vehicle for the addition of glycerol U.S.P. at concentrations of 5 per cent and 10 per cent.

The view that slow freezing is less damaging than that resulting from a rapid drop in temperature as originally advanced by Breedis (1942) was examined in relation to 12–13 day chick embryonic skin and leg muscle suspended in Gey's balanced salt solution, and in Gey's with 5 per cent and 10 per cent glycerol. Potentiometer recordings of the course of freezing and thawing

these solutions without tissue are given in figure I, while results for experiments 714–716 are given in figure II. The drop in temperature to —50° C. was achieved in six steps with a period of 15 minutes between each step. A parallel series of temperature changes was followed for the process of thawing (lower part of Fig. II). Results obtained after cultivating the explants for 6 days in roller tubes are represented in table 1. No evidence of cell survival was found for skin and muscle explants frozen in Gey's

TABLE 1

Effect of Glycerol in Relation to Gradual Freezing and Thawing of Tissues from 13-Day Chick Embryos

Freezing Medium	Expt. No.	Gradual Freezing (to—50° C.) and Thawing Survival Ratio		Survival Ratio
5 per cent Glycerol (in Gey's BSS)	714	Skin-epithelium 0/20;	spindle cells	16/20
		Muscle fibers 18/20;	spindle cells	20/20
10 per cent Glycerol (in Gey's BSS)	715	Skin-epithelium 0/20;	spindle cells	15/20
		Muscle fibers 20/20;	spindle cells	20/20
Gey's BSS	716	Skin — TOTAL INHIBITION		
		Muscle — TOTAL INHIBITION		

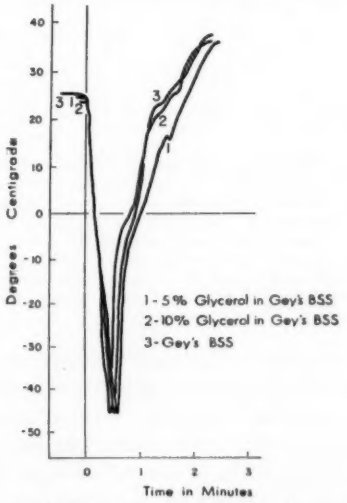

FIG. I. Record of rapid freezing and rapid thawing with a thermocouple in each of three test solutions without tissue fragments:
Curve 1. 5 per cent glycerol in Gey's balanced salt solution.
Curve 2. 10 per cent glycerol in Gey's balanced salt solution.
Curve 3. Gey's balanced salt solution.

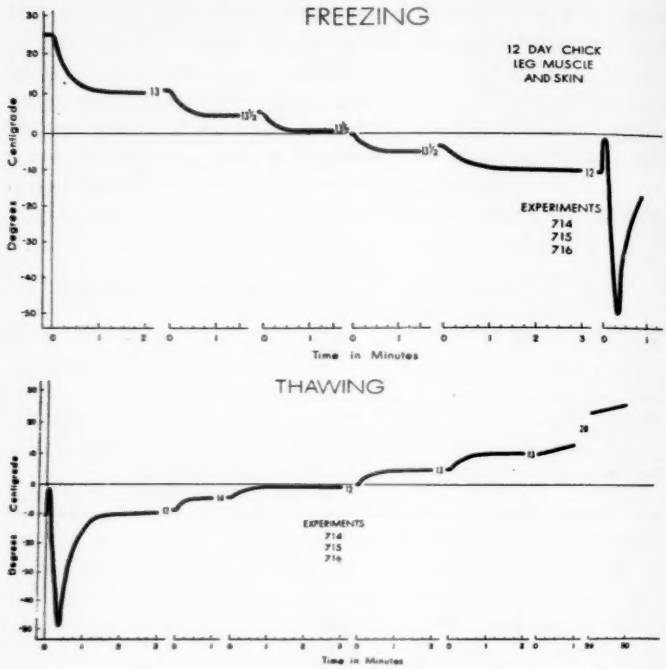

FIG. II. Record of freezing and thawing by stages (expts. 714, 715, 716) from room temperature to –50° C. Time between each immersion was 15 minutes. Recording thermocouple was placed in a tube with Gey's balanced salt solution not containing tissue fragments. In order to condense the record, constant or slightly changing temperature levels are represented as breaks with the time for each interval given in minutes.

The course of the freezing process is shown in the upper half of the graph. Continuation of the record during the thawing process is given in lower half of the figure.

solution alone. Epithelial cells did not appear to be protected by either 5 per cent or 10 per cent glycerol, although three-quarters of the explants produced relatively rich outgrowths of spindle cells. Muscle tissue proved fully resistant to the thermal changes, little difference being found between the value of 5 per cent and 10 per cent glycerol. However, as previously noted (Nakai, Pomerat, and Gilliland, 1954), with a slight retardation of spindle cell emigration the multinuclear fibers were relatively more luxuriant than those generally observed in such cultures.

While chick muscle had been found to be more easily damaged than skin as a result of rapid freezing and thawing (Pomerat and Lewis, 1953a), the reverse relationship was found with regard to heat injury (Nakai, Pomerat, and Blocker, 1954).

The effect of rapid freezing to —50° C. followed immediately by rapid thawing to room temperature was studied in relation to the protective action of glycerol in experiments 702–707 (Fig. III). Results are summarized in table 2 which shows that freezing in Gey's balanced salt solution without glycerol (expt. 704) completely inhibited all outgrowth from both skin and muscle explants, while corresponding unfrozen fragments (which served for all series in experiments 702–716) produced characteristic luxuriant cultures during the standard 6-day incubation period. Epithelium was found in 12 out of 20 explants treated with 5 per cent glycerol and in 7 out of 20 explants frozen in the 10 per cent glycerol solution. In view of the variation in the amount of epithelium present from explants of both series, the difference which might be due to the two glycerol concentrations was not believed

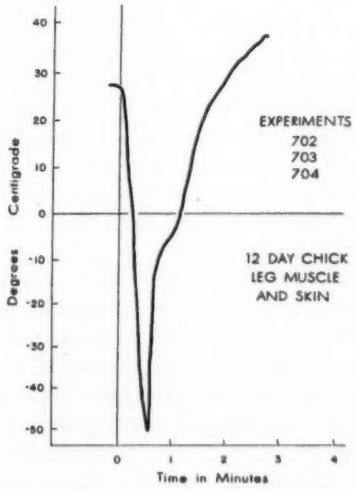

FIG. III. Record of rapid freezing from room temperature to –50° C. and rapid thawing in a bath at +37° C. (expts. 702, 703, 704). The recording thermocouple was placed in the core of the tissues in the fluid medium of experiment No. 702.

TABLE 2

Effect of Glycerol on the Survival of Cells from 11-Day Chick Tissues Following Rapid Freezing and Thawing

Rapid Freeze (to —50° C.) and Thawing				Unfrozen Controls		
Freezing Medium	Expt. No.	Tissue Outgrowth	Survival Ratio	Expt. No.	Tissue Outgrowth	Survival Ratio
5 per cent Glycerol (in Gey's BSS)	702	Skin-epithelium	12/20	705	Skin-epithelium	20/20
		Muscle fibers	20/20		Muscle fibers	19/19
10 per cent Glycerol (in Gey's BSS)	703	Skin-epithelium	7/20	706	Skin-epithelium	20/20
		Muscle fibers	20/20		Muscle fibers	18/20
Gey's BSS	704	Skin-epithelium	0/20*	707	Skin-epithelium	19/20
		Muscle fibers	0/20*		Muscle fibers	20/20

* + = No spindle cells. These elements were found in all other cultures in the table.

to be significant. Cultures of leg muscle produced excellent outgrowth of multinuclear fibers both in the 5 per cent and 10 per cent glycerol freezing mixtures.

Storage in glycerol at temperatures sufficiently low to reduce metabolism to an absolute minimum has now been well proven to assure the viability of spermatozoa and various tissue cells. However, it was thought important to add data on the protective action of glycerol after low temperature exposure followed by storage at usual ice box and somewhat lower temperatures.

The potentiometer record of the freezing and cooling of chick explants used for this purpose is shown in figure IV and the results obtained are summarized in table 3. Outgrowth was obtained only for muscle explants under three conditions: Following freezing in 5 per cent glycerol and storage at +1° C. to +6° C. (ice box) in which muscle fibers were found in 10 out of 20 explants (expt. 711) and after freezing in 10 per cent glycerol with storage at ice box or deep freeze temperature (—16.5° C. to —23.3° C.), under which conditions more than half of the explants produced typical muscle fibers (709, 712). In all three series showing muscle fibers spindle cell outgrowth was present but less abundant than that of standard controls. These results are in keeping with those reported by Scherer and Hoogasian (1954) who found better preservation for both the L and HeLa strains at —60° to —70° C. than at —20° C.

B. *The Effect of Glycerol on Cell Organoids*

Epithelial cells in cultures of adult mouse kidney and liver from newborn rats were employed by virtue of their large size

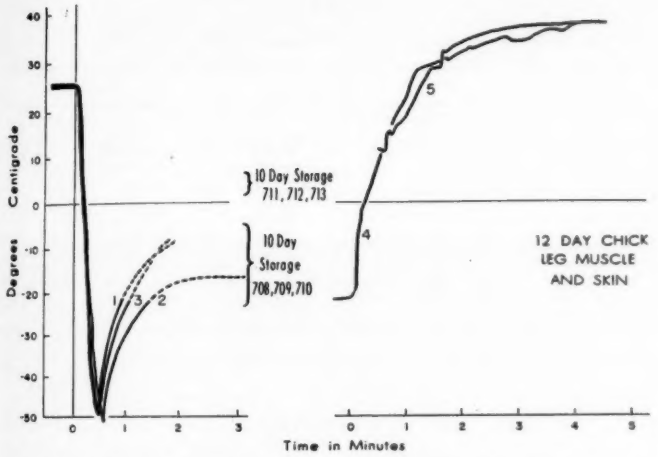

FIG. IV. Record of rapid freezing from room temperature to –50° C. and partial thawing at room temperature just before storage:

Curve 1. No. 708 and No. 711 in 5 per cent glycerol in Gey's BSS.

Curve 2. No. 709 and No. 712 in 10 per cent glycerol in Gey's BSS.

Curve 3. No. 710 and No. 713 in Gey's BSS.

Dashed extensions of the curves represent temperatures of experiments 708, 709 and 710 in deep freezer storage just prior to stabilization of the temperature.

Thawing of stored tissues 10 days later by immersion in a bath at +37° C:

Curve 4. No. 708, 709, 710.

Curve 5. No. 711, 712, 713.

All recordings shown in figure III were made with the thermocouple in a tube with Gey's balanced salt solution not containing tissue fragments.

to observe the effect of glycerol at various concentrations and time intervals on organoids. Cultures were made on cover glasses and placed in roller tubes "back to back," as described by Pomerat and Lewis (1954), using an avian plasma clot and a fluid nutrient medium consisting of 50 per cent human ascitic fluid, 45 per cent Gey's balanced salt solution and 5 per cent chick embryonic extract.

Kidney epithelium was obtained from adult mice of the C57 strain. Typical results shown in plates I and II were obtained with 7-day roller tube cultures photographed with dark phase contrast microscopy (AO). Cells in untreated control cultures

TABLE 3

Effect of Glycerol on the Survival of Cells from 12-Day Chick Embryonic Tissues Frozen to —50° C., Then Stored at Deep Freezer and Ice Box Temperatures

	Stored 10 Days at —16.5° C. to —23.3° C.			Stored 10 Days at +1° C. to +6° C.		
Freezing Medium	Expt. No.	Tissue Outgrowth	Survival Ratio	Expt. No.	Tissue Outgrowth	Survival Ratio
5 per cent Glycerol	708	Skin	0/20	711	Skin	0/20
(in Gey's BSS)		Muscle	0/20		Muscle fibers*	10/20
10 per cent Glycerol	709	Skin	0/20	712	Skin	0/20
(in Gey's BSS)		Muscle fibers*	16/20		Muscle fibers*	12/20
Gey's BSS	710	Skin	0/20	713	Skin	0/20
		Muscle	0/20		Muscle	0/20

* Spindle cells in 20/20. No spindles from explants of any other series.

were arranged in closely packed sheets with well defined boundaries (Pl. I, 1). Nuclei were generally slightly oval with well defined membranes and contained several nucleoli, one of which was usually quite large. The cytoplasm was rich in filamentous mitochondria and the perinuclear zone contained numerous granules, probably representing lipoprotein droplets. In our illustration (Pl. I, 1), the central cell was selected to show the granules while the surrounding elements, in a slightly different plane of focus, revealed mitochondrial patterns. All figures in plate I were photographed 5 hours after the experiment had been launched, while those in plate II show corresponding cells maintained at 37° C. and recorded 24 hours later. Figure 5 in plate II demonstrates that in cells of untreated controls little change had occurred in the organoids. After 5 hours, in the presence of 5 per cent glycerol (Pl. I, 2) incorporated in the fluid nutrient medium, the epithelial elements appeared somewhat shrunken and filamentous mitochondria could not be found, although there seemed to be a considerably larger number of fine granules in the cytoplasm. No marked change was produced from this condition following 24 hours' residence in 5 per cent glycerol. In addition to the disappearance of filamentous mitochondria, cells of corresponding cultures maintained for 5 hours in 10 per cent glycerol (Pl. I, 3) showed indistinct cell boundaries and a tendency toward nucleolar vacuolation. Cultures remaining in this

medium for 24 hours showed marked cell shrinkage, peripheral blisters, larger cytoplasmic vacuoles, and shrunken nuclei with dense round nucleoli (Pl. II, 7). The phenomenon of cell shrinkage with associated packing of cytoplasmic constituents was so marked in elements exposed to 20 per cent glycerol for 5 hours that epithelial sheets were almost completely destroyed (Pl. I, 4). Optically dense accumulations of cytoplasm and spine-like

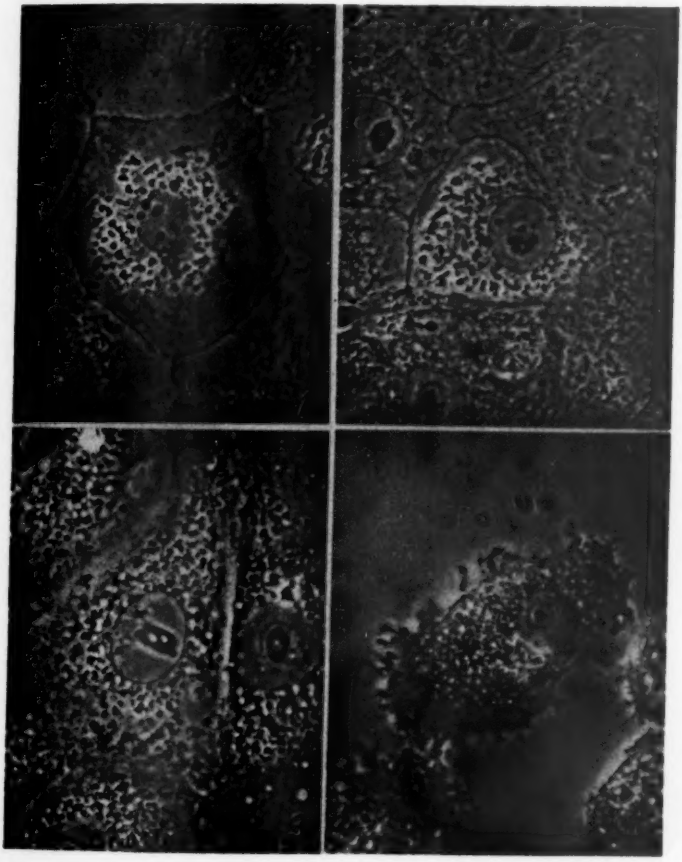

PLATE I

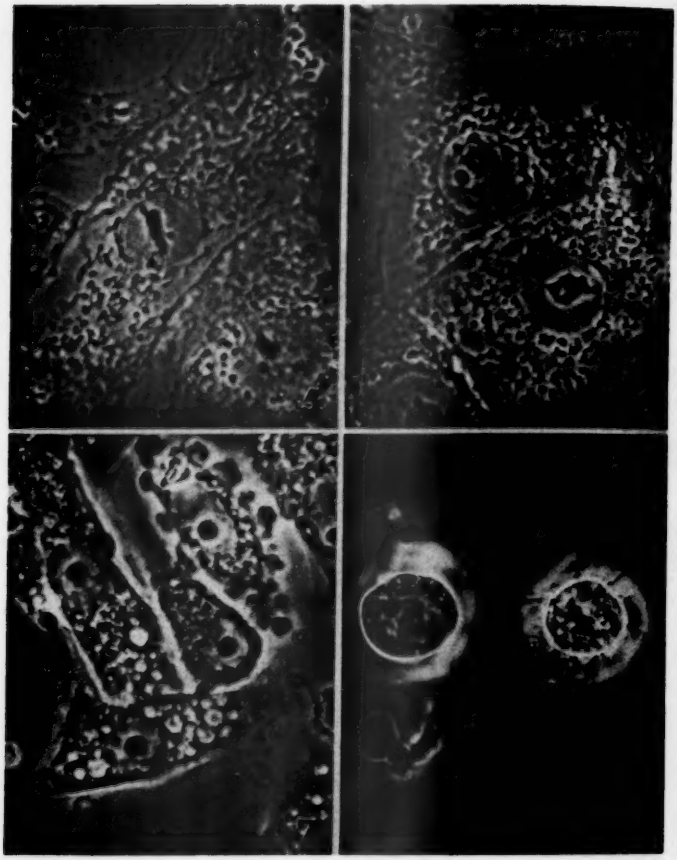

PLATE II

processes were common at the periphery of these cells. Twenty-four hours later epithelium in 20 per cent glycerol consisted of small, free rounded cells occasionally having long radiating threads of cytoplasm (Pl. II, 8).

Experiments with 13-day cultures of epithelium from the liver of newborn rats gave results closely paralleling those reported for the mouse kidney, but there appeared to be somewhat greater

persistence of mitochondria to the action of 5 per cent glycerol at the end of the 5 hour period.

C. *The Effect of Glycerol on Cell Activity*

It was obvious from the study of epithelial reactions to glycerol with photomicrographs as illustrated by plates I and II that marked changes in activity could be expected of cells in a perfusion chamber (Pomerat, Lefeber, and Smith, 1954) under similar experimental conditions with the aid of phase contrast, time-lapse cinematographic records.

Approximately 600 feet of 16 mm. film records were made of epidermal cells cultivated in hanging drop preparations (*cf.*, Lewis, Pomerat, and Ezell, 1949) from adult human split skin obtained in the course of grafting procedures. Fields were selected at the margin of outgrowth of cultures mounted in a perfusion chamber. The activity of approximately 15 to 30 cells was followed with the use of an 8 mm. dark phase objective in the absence of an ocular at a speed of 8 frames per minute. After establishing a base line record for one to one and one-half hours as a reference control for the behavior of the cells in the nutrient fluid used in the culture system, records of each change of perfusate were made for approximately one hour except where otherwise noted.

In the first film series, the glycerol percentages by volume in the nutrient fluid were changed from 5, 10, 20, 50, 80, 100, 80, 50, 20, 10, 5, and 0. Epidermal cells were derived from a 7-day culture. In a perfusate without glycerol, marginal elements showed rhythmic extrusion and withdrawal of cytoplasm. Characteristic ruffles, formed in the ectoplasmic portion, led to the formation of vacuoles in the inflowing activity which has been described by the term "pinocytosis" (cell drinking). With the introduction of 5 per cent glycerol, the cytoplasm of all marginal elements was slightly withdrawn but within a few minutes this reaction gradually was reversed and pinocytosis restored. The retreat of outwandering elements was induced again with the perfusion of 10 per cent glycerol. This led to the separation of individual elements at the periphery of the culture, but with the retention of narrow connecting strands which were optically dense. The cells progressively became rounded while showing

marked "boiling" at the surface. At the end of the hour run, 20 per cent glycerol was added. Granules in the cytoplasm continued to show movements and boiling persisted, but peripheral cytoplasmic extrusions did not reappear. The addition of 50 per cent glycerol led to the cessation of movement of the larger cytoplasmic granules. In 80 per cent glycerol, clear blisters arose from the surface of all of the cells in the field. No further change was observed with the use of 100 per cent glycerol. The process was then reversed with exposure to 80 per cent, 50 per cent, 20 per cent, 10 per cent, 5 percent and the total elimination of glycerol. The cells did not recover but the clear blisters were progressively reduced until the system appeared fixed without clear vacuolar projections at the periphery.

On the basis of this experience, similar moving picture records were made of two other cultures of human epidermis. These had been incubated for 4 days. Following the establishment of a base line in the control nutrient fluid for one hour and 20 minutes, the perfusate was changed progressively to 10 per cent glycerol. After demonstrating the effect, the glycerol was removed in parallel gradual steps. Plate III, figures 1–4, summarize a cycle in the characteristic reactions of human epidermis to the presence of glycerol. In plate III, figure 1 representing zero time, the central cell at the margin of the outgrowth is seen to contain clear vacuoles representative of the process of pinocytosis. Eleven minutes after the addition of 2 per cent glycerol the marginal cytoplasm had withdrawn as seen in plate III, figure 2. At 36 minutes (Pl. III, fig. 3), the recession of the cells had reached its maximum. Progressively, the cytoplasm of epithelial elements was extruded so that 61 minutes later (Pl. III, fig. 4), the sheet of outwandering cells resembled that shown for zero time but along a line somewhat closer to the explant. With the addition of 4, 6, 8, and 10 per cent glycerol, the pattern of cellular withdrawal followed by almost complete recovery was repeated. The perfusion chamber containing 10 per cent glycerol was allowed to remain on the microscope stage overnight (16 hours) without taking additional photographs. The following morning, fresh 10 per cent glycerol in the same nutrient fluid as previously used was introduced. Some blistered cells showed fragmentation

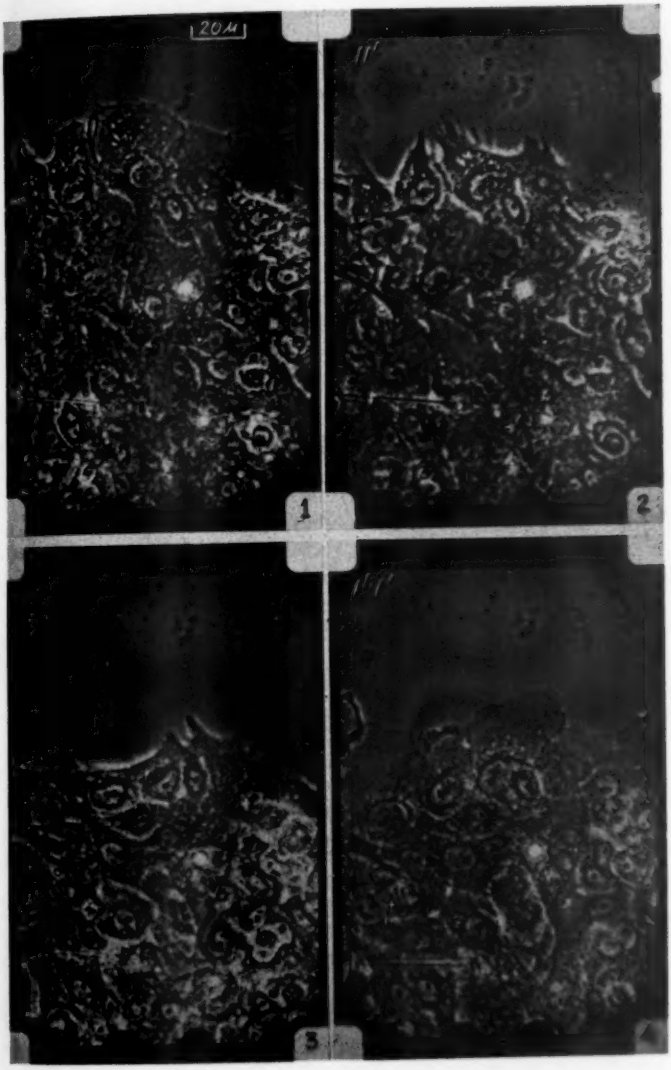

PLATE III

of distal processes. The addition of perfusates containing decreasing concentrations of glycerol (8, 6, 4, and 2 per cent) showed progressive recovery of cytoplasmic activity with an increase in the phenomenon of pinocytosis. It is believed that the rehydration of the system led to an intensification of the process of cell drinking. It is hoped that the problem of pinocytosis may be explored with the aid of this finding.

Plate IV represents the stages in the recovery of the epithelial cells used in the previous experiment as revealed by enlargements of the margin of outwandering elements. In each photograph the time is given in the upper left-hand corner, while the magnification for the entire series is given in the upper right-hand corner of the frame representing zero time. The appearance of the cells on the day following restitution of the system to the control fluid nutrient medium is given in the lower right-hand figure. With the exception of the first and the last photographs in the series shown in plate IV, each illustration shows the end of the recovery period following a change in composition of the perfusate (all of the photographs in this plate were enlarged from the 16 mm. cine record). Thus, the second photograph from the top of the left-hand column (1 hour) corresponds to the stage of recovery shown in figure 4 of plate III. Under the conditions of this experiment, more than half of the cells under study survived contact with the glycerol.

Summary

This study was designed to add information regarding the protective action of glycerol to cells subjected to freezing and thawing with the use of tissue culture methods.

Fragments of chick embryonic skin and of leg muscle in a fluid nutrient medium containing 5 per cent and 10 per cent glycerol were found to resist rapid freezing and rapid thawing more successfully than that following a procedure employing six graded steps for both lowering and raising the temperature. With slow freezing the epidermal cells of the skin were totally inhibited, while most of the explants showed relatively rich outgrowths of spindle cells. Approximately half of the skin explants exposed to rapid freezing and thawing produced outwandering epithelial cells. Multinuclear muscle fibers proved resistant to

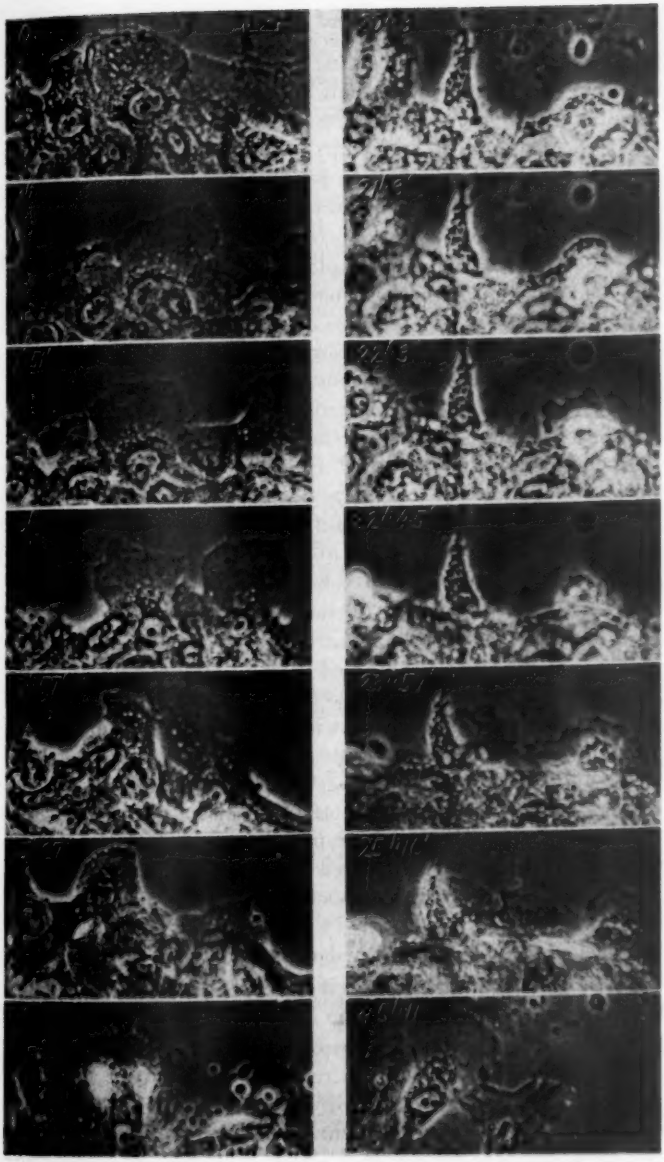

PLATE IV

both stepwise and rapid freezing and thawing. Their predominance in such cultures appeared to be reflected by moderate retardation of spindle cells. In contrast to those in both 5 per cent and 10 per cent glycerol, explants frozen either slowly or rapidly in Gey's balanced salt solution gave no evidence of viable cells following 6 days' incubation in roller tube culture.

Following rapid freezing to —50° C. and rapid thawing in 5 per cent glycerol, storage of explants at from +1°.C. to +6° C. for 10 days was more favorable for the persistence of viable muscle fibers than was storage at temperatures ranging from —16.5° C. to —23.3° C. for a similar period. Leg muscle maintained under similar conditions but in 10 per cent glycerol withstood storage at the two temperatures almost equally well, but the resulting outgrowth of muscle fibers was not as rich as that obtained following rapid freezing and thawing without storage. No viable cells could be demonstrated from cultures of skin stored at the two temperature levels in either 5 per cent or 10 per cent glycerol. Cells in all explants frozen in the Gey's balanced salt solution appeared to be irreversibly injured.

Epithelium from 7-day cultures of adult mouse kidney and of newborn rat liver exposed to various concentrations of glycerol in nutrient fluid were examined in the living state with phase microscopy after 5 hours and 24 hours. Mitochondrial filaments were not evident in cells following contact for 5 hours with 5 per cent glycerol. Vacuolated nucleoli and indistinct intercellular boundaries in the presence of 10 per cent glycerol recorded at 5 hours showed dense nucleoli and the separation of individual cells with large blobs at their periphery after 24 hours. Epithelial sheets were disorganized with pericellular evidence of injury following 5 hours' contact with 20 per cent glycerol in the nutrient medium. After 24 hours such cells were small, rounded and showed other evidences of marked injury.

Phase contrast, time-lapse cine records were made to show the effect of bringing glycerol in contact with human epidermal cells in a perfusion chamber assembly. After exposure to 5 per cent glycerol, moderate injury resulted from contact with a 10 per cent solution and marked injury with 20 per cent glycerol. Cessation of granular movement in rounded cells was observed in the presence of 50 per cent glycerol. After confronting this preparation with 80 per cent glycerol and removing the glycerol

by progressive steps, no evidence was obtained of cell survival under the conditions of the experiment.

The course of injury and recovery to epidermal cells exposed to changes in the per cent concentration of glycerol from 2, 4, 6, 8, 10, 8, 6, 4, 2 and zero at approximately one hour intervals was analyzed with cine records. The cultures remained overnight in the presence of 10 per cent glycerol but without being exposed to the microscope light. It is estimated that approximately 50 per cent of the epithelial cells survived this treatment. Pinocytosis appeared to be intensified during the process of glycerol removal.

Acknowledgment

Grateful acknowledgment is made to Mr. George Lefeber and Mr. Earl Pitsinger for technical assistance with photographic work. Thanks are extended to Mr. Charles Bishop for the preparation of graphs.

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FURTHER STUDIES ON SANGUININ, A BLOOD DERIVATIVE

DOROTHY M. WHITNEY AND MARIO PAVAN

Sanguinin, a derivative of bovine blood, has been shown to be biologically active by antibacterial properties (Anigstein *et al.*, 1950; Whitney *et al.*, 1950; Micks *et al.*, 1951), by wound healing effects in chronic ulceration in man (Nilasena *et al.*, 1954; Mullins *et al.*, 1955), and in experimental surgery (Bader, 1952). These biological activities of Sanguinin justify its classification among antibacterial substances of animal origin which were for the past several years the object of study by one of us (M. Pavan). These studies were mainly directed to the chemical purification of biologically active substances isolated from secretions and extracts of various lower animals, arthropods, worms, amphibia, reptiles, etc. (Pavan, 1948; 1952; 1955). The present work carried out at the University of Pavia was centered on the purification of the biologically active principle of Sanguinin using the inhibition of bacterial growth as the index of activity. Initial experiments were designed to determine the survival of antibacterial activity of Sanguinin in a group of solvents, followed by a series of experiments to determine whether the antibacterial principle could be separated from the pigmented soluble portion of Sanguinin.

Materials and Methods. Sanguinin, prepared at The University of Texas Medical Branch, is a substance derived from bovine blood by means of hydrolysis with proteolytic enzymes (Anigstein *et al.*, 1950; Whitney *et al.*, 1950). Some interesting comments were made by J. K. Cline (1951) as to anti-liver catalase activity of Sanguinin which was found to be effective in lowering liver catalase in C57 and C 3H mice in a 24 hour period.

For the antibacterial studies, the test organism was B. *subtilis*

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from the stock cultures of the University of Pavia. The technique in demonstrating antibacterial activity was the agar well technique under standard conditions. The organism was grown in nutrient broth at 37° C. for 24 hours then inoculated into melted and cooled nutrient agar which was allowed to harden and the wells made by removing cores of agar with standard penicillin cups.

Extracts of Sanguinin in various solvents in 0.1 ml. amounts were placed in the wells and results recorded by observing sizes of inhibition areas around the wells after incubation at 37° C. for 24 hours.

The solvents were chloroform, aqueous sodium citrate 3.8 per cent, physiological saline, ethanol, methanol, acetone, benzene, petroleum ether, ethyl ether, acetic ether (ethyl acetate), carbon disulfide, carbon tetrachloride and distilled water. One ml. solvent was used to which 2 mg. Sanguinin was added. Time for extraction of the active soluble substance was usually 24 hours at room temperature. Considering the antibacterial effect of certain of these solvents appropriate controls were made with no Sanguinin added. Furthermore all supernates, including controls, were filtered through cotton to remove traces of sediment, air dried at 40° C., and the dry residue dissolved in distilled water to be tested for antibacterial activity.

Decolorizing agents were animal carbon, infusorial earth, aluminum oxide, cellulose acetate, pulverized agar (2 to 15 mg. decolorizing agent per 1 ml. solvent containing 2 mg. Sanguinin). At the end of 2 hour adsorption period at room temperature, tests for antibacterial activity were made.

Experimental Results. It was found that the best solvents in which antibacterial activity could be demonstrated were distilled water, aqueous sodium citrate 3.8 per cent, physiological saline and methanol. In each of these 4 solvents, however, solubility was not complete as evidenced by slight sediment at the bottom of the tubes. Each dark brown supernate showed antibacterial activity against B. *subtilis* in the concentration used (2 mg. Sanguinin to 1 ml. solvent). On the other hand the completely colorless supernates of Sanguinin in acetone, benzene, ethyl ether[1] petroleum ether, acetic ether, carbon tetrachloride

[1] Dr. Wiktor W. Nowinski, Director of Tissue Metabolism Laboratory, The University of Texas Medical Branch, was kind enough to perform a pro-

and carbon disulfide were inactive against bacteria. Likewise the weakly colored supernates of Sanguinin in ethanol and chloroform showed no antibacterial activity. In regard to stability of the antibacterial principle in saline, water, methanol and sodium citrate, the activity was unchanged after standing in these solvents at room temperature for 14 days. Furthermore the antibacterial principle in these solvents will resist drying at 40° C. in air currents leaving an active principle in the water soluble residue.

During these experiments, the dark brown aqueous solutions of Sanguinin when placed in wells of the agar plates seeded with B. *subtilis*, formed a halo of inhibition. This halo corresponded in part with the zone of diffused pigment, but in the peripheral part, inhibition of growth extended beyond the pigmented portion. This observation which seemed to indicate that the antibacterial factor may not be entirely associated with the pigment of the extract, led to the next series of experiments on the adsorption of pigment of Sanguinin in solution to various decolorizing agents, and to attempts to separate a colorless fraction showing antibacterial activity.

Of the 5 decolorizing agents used, animal carbon, infusorial earth, aluminum oxide, cellulose acetate and pulverized agar, only animal carbon adsorbed the pigment from the deeply colored extracts of Sanguinin in distilled water, saline, sodium citrate and methanol. As shown in table 1, the antibacterial activity was highest in dark brown solutions to which no carbon was added especially in methanol, sodium citrate, and water extracts. Addition of 2 mg. carbon per ml. Sanguinin extract, adsorbed much of the pigment, as shown by slight loss of antibacterial activity. Addition of 4 mg. carbon per ml. Sanguinin extract resulted in weakly colored supernates showing considerable activity in water and sodium citrate solutions while methanol extract so treated had lost most of its color, but still showed some activity. When 8 mg. carbon per ml. was added to saline, citrate and water extracts, the pale yellow solutions were found to have about half the activity shown in controls with no carbon added. The almost colorless solutions resulting from use of 10 mg. carbon per ml. extract in saline, water, and citrate showed

longed extraction of Sanguinin in Soxhlet apparatus with ether which yielded a fair amount of ether soluble fraction.

TABLE 1

Bacterial inhibition (*B.* subtilis) *by 0.2 Per Cent Sanguinin in Various Solvents in Relation to Pigment Adsorption to Animal Carbon*

Animal Carbon mg./ml.	Sanguinin in Methanol	Sanguinin in Saline	Sanguinin in Sodium Citrate 3.8 Per Cent	Sanguinin in Distilled Water
0 (control)	RB* 4+†	DB 3+	DB 5+	DB 4+
2	LB 2+	LB 3+	LB 4+	MB 3+
4	AC 1+	LB 2+	LB 3+	LB 3+
8		Y 2+	Y 2+	Y 2+
10		AC tr	AC tr	AC tr
15	C 1+	C 0	C 0	C 0

* RB red brown
DB dark brown
MB medium brown
LB light brown
Y yellow
AC almost colorless
C colorless
tr trace of color

† Degree of inhibition is expressed by symbols 4+ to 0 the former indicating the largest area of inhibition.

only traces of antibacterial activity. Contrary to the negative results obtained in the other colorless extracts when 15 mg. carbon per ml. extract was used, this same quantity added to methanol extract resulted in a completely colorless solution showing a definite antibacterial activity. Controls in solvents without Sanguinin when dissolved in water after drying, showed no antibacterial activity.

Summary. 1. These experiments indicate that one step in the purification of the antibacterial fraction of Sanguinin consisted of dissolving Sanguinin in methanol, drying the supernate and dissolving the residuum in water, a process which does not impair antibacterial activity. This process alone could be considered a partial purification of the complex compound after inert substances insoluble in methanol are removed.

2. Experiments on adsorption indicate that it is possible to isolate a colorless substance from crude Sanguinin showing antibacterial activity by using methanol as solvent and varying amount of carbon and time of adsorption. Whereas purification of Sanguinin was evaluated by antibacterial activity, the effect of these procedures upon cellular response such as wound healing remains yet to be investigated.

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PROCEEDINGS OF THE TEXAS BRANCH OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

ABSTRACTS

Of Some of the Papers Read at the Fall Meeting of the
Texas Branch, Society of American Bacteriologists
October 28-29, 1955
The University of Texas
Southwestern Medical School
Dallas, Texas

Enzymatic Induction in an Algal Flagellate. (Comparison with that of Yeasts and Bacteria). Vincent P. Cirillo, Department of Preventive Medicine and Public Health, University of Oklahoma School of Medicine, Oklahoma City.

The fatty acid oxidase (FAO) activity of the algal flagellate, *Polytoma uvella*, depends upon an inducible enzyme system. The level of FAO, measured as the rate of butyric acid oxidation, is from 600–700 per cent higher in butyrate-grown cells than in acetate grown cells. After 24 hours subculture in an acetate medium, the FAO activity drops to the "non-adapted" level.

Increases in FAO activity can be induced in non-proliferating, acetate-grown cells by incubation with butyric or caproic acid in the presence of an exogenous nitrogen source. In the presence of 50 mg. per cent NH_4CL a 500–600 per cent increase in enzyme activity occurs in from 3 to 6 hours, depending upon the age of the cells. The rate of FAO induction is greatest in cells taken from the logarithmic phase of growth. In log phase cells enzyme synthesis is linear, starting after a 25–30 minute induction period. The kinetics of induction and synthesis are variable in older cells.

Ultraviolet irradiation (at 2540Å) selectively inhibits FAO induction at intensities which do not inhibit the activities of either the constitutive acetic acid oxidase or previously induced fatty acid oxidase.

The Pigment of an Orange Mutant of Serratia marcescens. J. A. Green and R. P. Williams, Department of Microbi-

ology, Baylor University College of Medicine, Houston 25, Texas.

The natural pigment of the microorganism, *Serratia marcesens*, is red. By ultraviolet irradiation of a suspension of red organisms, we have produced a mutant with orange pigment. The latter pigment has been characterized by chromatography, ultraviolet, visible, and infrared spectra, and by determining the nitrogen and chloride content of the hydrochloride salt. These various determinations established that the orange mutant pigment is related to, but different from, the natural pigment. Molecular weight determinations carried out by the Rast method indicated a probable value of 375. Of interest was the fact that the orange mutant produced a diffusible substance that caused white mutants of *Serratia* to become red. The red pigment so produced was identical to the natural red pigment.

The Chelating Properties of Prodigiosin Components. R. P. Williams, J. A. Green, and D. A. Rappoport, Departments of Microbiology, Biochemistry and Radiology, Baylor University College of Medicine, Houston 25, Texas.

Spectrochemical analysis of prodigiosin demonstrated the presence of polyvalent metals in the pigment fractions. Experiments carried out using isotopic Fe^{59} and Ca^{45} demonstrated that both of these metals were incorporated into prodigiosin. On the basis of radioactive isotopic counts, the pigment contained about twenty times as much iron as calcium. Fractionation of the pigment into its components established the fact that calcium is incorporated into both the blue fraction and combined red fractions to about the same degree. On the other hand, iron is incorporated into the blue fraction about six times as much as it is incorporated into the combined red fractions.

Microbial Synthesis of High Molecular Weight Hydrocarbons from Natural Gas. J. B. Davis, Magnolia Petroleum Co., Field Research Laboratories, Dallas, Texas.

The cell lipid fraction of gaseous hydrocarbon utilizing bacteria, actinomyctes and filamentous fungi contains small amounts of heavy saturated hydrocarbons. Organic soil deposits known as "paraffin dirt" (waxy appearance) found associated with certain

natural gas seeps have been essentially duplicated in the laboratory by flowing natural gas and air for eighteen months through soil. Natural gas flow was ceased and aeration continued for six months. The non-hydrocarbon lipid rapidly decreased, but the heavy saturated hydrocarbons remained relatively resistant to oxidation. Soils generally contain microflora capable of converting natural gas into organic matter containing small quantities of high molecular weight hydrocarbons.

The Effect of Preincubation on Chloramphenicol Resistance of Micrococcus pyogenes. T. E. Wilson and H. H. Rammsey, Department of Bacteriology, The University of Oklahoma School of Medicine, Oklahoma City.

Yeast extract and other complex materials counteract the inhibitory effect of chloramphenicol on *M. pyogenes.* This effect is probably due to a direct antagonism between the complex matrials and the antibiotic, and also to the growth-stimulating properties of the substances.

Experiments using membrane filters have shown that preincubation of cells in the absence of chloramphenicol prior to exposure to the antibiotic also endows them with a higher degree of resistance. Preincubation for as short a period as thirty minutes results in a significant increase in survivors when exposed to the antibiotic. This increased resistance is higher, proportionately, than is the increased resistance due to yeast extract.

The effect of preincubation is additive to the effect of yeast extract; whether or not the mechanisms are similar in both instances is unknown.

The Role of Antibiotics in Thermal Injury. I. Evaluation of Results of Antibiotic Sensitivity Tests During a Five-Year Period. Jose A. Rivera, Major, MSC, U.S.A.; Curtis P. Artz, Lieutenant Colonel, MC, U.S.A.; and Anthony Contreras. Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas.

The work on antibiotic sensitivity tests reported here comprises comparative studies of results obtained with these tests during a period of five years. Each organism isolated in cultures from patients on a Burn Ward was submitted to antibiotic sensitivity

tests. For practical purposes, however, only the more predominant pathogens, or potential pathogens, are considered at this time.

The predominant organisms, in the order of their frequency, were: *Micrococcus pyogenes* (*Staphylococcus aureus*, coagulase positive), *Pseudomonas*, *Proteus*, and *Streptococcus*, beta hemolytic.

Studies of results for the complete period prove to be of statistical significance.

Observations are discussed on changing antibiotic sensitivity patterns in infections of burned patients during treatment with penicillin, chlortetracycline, oxytetracycline, Streptomycin, chloramphenicol, neomycin, and polymyxin B.

Important factors that must be taken into consideration in evaluating and comparing different methods for testing sensitivity to antibiotics are stressed in this paper.

The Effect of H. pertussis Organisms on Macrophages in Tissue Culture. Harriet M. Felton and W. R. Stinebring, Department of Bacteriology and Parasitology, The University of Texas Medical Branch, Galveston, Texas.

The effect of *H. pertussis* organisms on fetal and kitten brain in tissue culture has been described. Other cell systems which are more readily available have been suggested for this type of investigation. Observation of guinea pig macrophages in contact with suspensions of live *H. pertussis* organisms were recorded by time lapse phase contract cinematography. The control preparation using supernatant solution without bacteria was observed in the same manner. The preparation containing the bacteria showed only occasional viable macrophages three hours after exposure. In the control preparation there was no evidence of any type of damage to the cells. The same suspension of *H. pertussis* which had been held at room temperature for eighteen hours introduced into another preparation in the same manner produced signs of damage in the macrophages after three hours but in a less marked degree.

A Report of Some Preliminary Studies of Brucella abortus Organisms Using Time Culture Technics. Americo Pomales-

Lebrón* and Warren R. Stinebring.† From the Laboratory of the Department of Microbiology, School of Medicine, University of Pennsylvania.

The intimate relationship of long lived phagocytic cells and *Brucella abortus* are of necessity studied *in vivo* only with great difficulty. The ease with which guinea pig phagocytes can be obtained and maintained in fairly pure culture for reasonable periods of time seemed to offer a means of attacking this problem.

Guinea pig pertioneal exudate cells were obtained by (1) injecting a dilute glycogen solution intraperitoneally, (2) washing out the peritoneal cavity with Hank's solution, (3) centrifuging down the cells from the suspension, and (4) resuspending the cells in a proper amount of 30 per cent normal Guinea pig serum—70 per cent Hank's solution. The cells were then planted in Porter flasks containing flying coverslips. *Brucella abortus* organisms were inoculated into the flasks and the cells were allowed to settle and ingest the bacteria. After a period of two hours the supernate was removed and new medium containing 10 micrograms of streptomycin was added. This was known to be sufficient to kill all extracellular brucella. Flying coverslips were harvested from flasks at various times and counts of the number of brucella per cell and of the number of monocytes per $mm.^2$ were done. The monocytes remaining in the flask were scraped off and used to determine the number of viable brucella per flask.

The following results were obtained: (1) there was definite evidence of intracellular multiplication of the organisms, (2) this growth was relatively slow and about two days was required before the monocytes became packed with bacteria, (3) the monocytes seemed to tolerate the organisms fairly well even after moderate multiplication had occurred.

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†At present McLaughlin Fellow in Infection and Immunity at the University of Texas, Medical Branch, Galveston.

CALLING ATTENTION TO

Items of Possible Interest to Medicos

February, 1956

General: E Belt gives Logan Clendening Lectures on *Leonardo the Anatomist* (Univ Kansas Press, Lawrence, 55, 76pp, $2) . . . H Danby translates Book 10 of the Code of Maimonides, *The Book of Cleanliness* (Yale Univ Press, New Haven, 55, 645pp, $7.5) . . . John F. Fulton is appropriately honored on his 25th anniversary at Yale (*Yale J Biol Med* Vol 28, No 3, January, 1956) . . . G de Santillana brilliantly examines *The Crime of Galileo* (Univ Chicago Press, 55, 353pp, $5.75) . . . K Sax says there's going to be *Standing Room Only: The Challenge of Overpopulation* (Beacon Press, Boston, 55, 206pp, $3) . . . RA Smith considers detection & measurement of infra-red radiation (*Sci Month* 82/1/56) . . . W Weaver well relates science to people (*Science* 122/1255/Dec 30/55) . . . D Woelfe edits debate on extrasensory perception, with PW Bridgeman's important comment on probability (*Science* 123/7—19/Jan 6/56).

Clinical: RD Adams, D Denny-Brown & CM Pearson survey *Diseases of Muscle* (Hoeber, NY, 56, 571pp, $16) . . . AB Baker edits *Clinical Neurology* (Hoeber, NY, 56, 2100pp, 3 vols, $60) . . . M Brucer describes cesium 137 teletherapy apparatus (*Amer J Roentg Rad Therap Nucl Med*, 50th anniversary vol, 75/49/56) . . . MC Cobey considers *Postural Back Pain* (CC Thomas, Springfield, Il, 56, 78 pp, $2) . . . F de Nicola discusses differential diagnosis of clotting diseases (*Ergeb Inn Med* 6/1/55) . . . RM Dorcus is careful in editing *Hypnosis & Its Therapeutic Applications* (McGraw-Hill, NY, 56, 12 sect, $7.5) . . . LI Gardner discusses *Adrenal Function in Infants & Children* (Grune & Stratton, NY, 56, 263pp, $7) . . . E Lyon goes into *Virus Diseases & the Cardiovascular System* (Grune & Stratton, NY, 56, 242pp, $6) . . . MF Oliver & GS Boyd note ethinyl estradiol corrects abnormal circulating lipid & lipoprotein concentrations following myocardial infarction (*Circulation* 13/82/56, issue dedicated to retiring editor TM McMillan) . . . SC Reed helps with *Counseling in Medical Genetics* (Saunders, Phila, 56, 268pp, $4) . . . IC Rubin & J Novak offer *Integrated Gynecology* (McGraw-Hill, NY, 56, 3 vols, 1922pp, $60) . . . OH Wangensteen rates anniversary issue of *Surgery*, with important report on post-operative aldosterone excretion & sodium retention by B Zimmerman & Co (*Surgery*, 39/161/56) . . . CV Weller reviews *Causal Factors in Lung Cancer* (CC Thomas, Springfield, Ill, 56, 113pp, $2.5).

Medical Sciences: EP Benditt & DA Rowley find chlorpromazine antagonizes 5-hydroxytryptamine (*Science* 123/24/Jan 6/56) . . . GL Curran & RL Costello report vanadyl sulfate in diet (0.05% $VOSO_4$) reduces hepatic cholesterol synthesis in rabbits & reduces excess aortic cholesterol (*J Exp Med* 103/49/56) . . . CJ Gubler discusses absorption & metabolism of iron (*Science* 123/87/Jan 20/56) . . . LH Louis & JW Conn show glycyrrhizinic acid (from licorice) acts like adrenal cortical steroids, which it resembles chemically, in causing NaCl & water retention (*J Lab Clin Med* 47/20/56)

. . . V Menkin reconsiders *Biochemical Mechanisms in Inflammation* (CC Thomas, Springfield, Ill, 56, 438pp, 2nd Ed, $6.5) . . . AH Morgan reveals *Kinships of Animals and Man* (McGraw-Hill, NY, 56, 848pp, $6.75) . . . G Oster & AW Pollister edit *Physical Techniques in Biological Research* (Academic Press, NY, Vol 1, *Optical Techniques*, 55, 564pp, $13.5; Vol 2, *Physical Chemical Techniques*, 56, 610pp $14; Vol 3, *Cells & Tissues*, 56, 622 pp, $14) . . . K Peach & MV Tracey edit *Modern Methods of Plant Analysis* (Springer, Berlin, 4 vols, 55–56, 2200pp, DM 400) . . . A Pi Suner (Trans by CM Stern) offers *Classics in Biology: A Survey of the Study of Life* (Philosophical Library, NY, 56, 322pp, $7.5) . . . FW Schueler shows ethanol groups yielding hemiacetals are respiratory depressants on relay centers (*J Pharmacol* 115/127/55) . . . MN Schwartz & Co discuss significance of heat-activated enzymes (*Science* 123/50/Jan 13/56) . . . M & D Schwimmer note *Role of Algae & Plankton in Medicine* (Grune & Stratton, NY 56, 96pp, $3.75) . . . L von Bertalanfy, as a biologist, looks at human nature (*Sci Month* 82/33/56) . . . WF von Oettingen offers 5 vol *Handbook of Toxicology*, Vol 1, *Acute Toxicities of Solids, Liquids & Gases* now ready (Saunders, Phila. 56, 408pp, $7) . . . M Woods & Co indicate chemotherapeutic possibilities of regulation of glucose utilization in tumors by stress-modified insulin-anti-insulin system (*J Nat Cancer Inst* 16/352/55).

MARCH, 1956

MOOD ALTERING DRUGS: EJ Algeri & AJ McBay find p-hydroxy-phenobarbital as etabolic excretory product of phenobarbital (*Science* 123/183/Feb 3/56). EV Evarts finds bufotenine & lysergic acid diethylamide cause sensory disorder & quietness in monkeys without loss of muscle power (*Arch Neurol Psychiat* 75/49/56). BF Hoffman & Co propose quantitative evaluation of sleep (*J Appl Physiol* 8/361/56). E Jacobsen & Y Skaarup find diethylamino-ethyl benzilate HCl (benactylzine, or "Suavitil") an anticholinergic sedative (*Acta Pharmacol Toxicol* 11/24/147/55) & I Munkvad notes usefulness in allaying irritation in neurotics without value in psychoses (*Acta Psych Neurol Scand* 30/729/55). R Mangold & Co study cerebral circulation & methabolism in sleep & prolonged wakefulness (*J Clin Invest* 34/1092/55). A Meister & PR Downey find vitamin B_6 participates in glutamine transaminase system & that isonicotinic acid hydrazide depletes B_6 body reserves (*Proc Soc Exp Biol Med* 91/49/56). WG Mitchell & RC Grubb find CO_2 inhibits audiogenic convulsions (*Science* 123/223/Feb 10/56). M Rushbrook & Co suggests ranking method in assessing hypnotics & find phenyl-ethyl-glutarimide ("Doriden") a reliable rapid hypnotic (*Brit Med J* 1/139/Jan 21/56). BC Schwarz & Co study behavioral & EEG effects of hallucinogenic drugs given intracisternally & find no antagonism between serotonin & lysergic acid diethylamide, which like adrenochrome & adrenolutin cause neuromuscular withdrawal after initial uneasy nausea (*Arch Neurol Psychiat* 75/83/56). AW Wise & Co note turnover of brain phospholipids is reduced by chlorpromazine (Ibid p 54). JZ Young ably reviews organization within nerve cells (*Endeavor* 15/5/56).

CLINICAL: P De Nicola ably summarized *The Laboratory Diagnosis of*

Coagulation Defects (CC Thomas, Springfield, Ill., 1956, 240pp, $6). EB Hay & R Huggins discuss hemodynamics of Na dehydrocholate in shock, by increasing arterial blood flows to liver (*Surg* 39/199/56). RJ Jones notes reduction of serum cholesterol after 30 Gms daily oral concentrated beef brain extract free from proteins (*J Lab Clin Med* 47/261/56). JJG Prick & Co reviews *Thallium Poisoning* (Cleaver-Hume, London, 1955, 154pp, 19s). MB Rosenblatt discusses *Cancer of the Lung: Pathology, Diagnosis & Treatment* (Oxford Univ Press, NY, 1956, 330pp, $15). S Standard & H Nathan ask *Should the Patient Know the Truth?* (Interscience, London, 1955, 160pp, 15s). LT Webster Jr & CS Davidson find carbonic anhydrase inhibitor "Diamox" may induce hepatic coma in susceptible alcoholics with cirrhosis (*Proc Soc Exp Biol Med* 91/27/56).

PRECLINICAL: JAV Butler reviews facts & theories on effects of ionizing radiation on biological materials (*Rad Research* 4/20/56). PW Durbin & Co (under JG Hamilton) find lanthanons not absorbed orally, but after parenteral injection fixed in bone & liver with very slow release from bone (*Proc Soc Exp Biol Med* 91/78/56). C Else & E Scharrer edit 35th Vol *Ergeb Anat Entwicklges* (Lange & Springer, Berlin, 1956, 554pp). KH Gustavson considers *The Chemistry & Reactivity of Collagen* (Academic Press, New York, 1956, 342pp, $8). AW Johnson & A Todd show complex porphyrin character of vitamin B_{12} (Endeavor 15/29/56). EA Kabat writes on *Blood Group Substances: Their Chemistry & Immunochemistry* (Academic Press, New York, 1956, 330pp, $8). E Kokas & Co find guanylic acid derivatives from embryonic heart & ATP increase muscular efficiency & inhibit cardiac & adrenal work hypertrophy (*Acta Physiol Acad Sci Hungaricas* 7/409/55). WD McElroy & B Glass edit *Symposium on Inorganic Nitrogen Metabolism: Function of Matallo-flavoproteins* (Johns Hopkins Univ Press, Baltimore, 1956, 728pp, $10). F Stohlman Jr & G Brecher find anemic plasma factor as erythropoietic stimulant in sublethal total body irradiation anemia (*Proc Soc Exp Biol Med* 91/1/56). F Wassermann discusses intercellular components of connective tissue (*Ergeb Anat Entwicklges* 35/254/56).

GENERAL: I Galdston edits *Ministry & Medicine in Human Relations* (International Univ Presses, NY, 1955, 173pp, $3.5). RM Lambert introduces memorial tributes to AM Pappenheimer 1878–1955 (*Lab Invest* 5/1–131/56). AV Neale edits Thomas Phaire's 1545 *Boke of Children*, the 1st English text in pediatrics (E & T Livingstone, Edinburgh, 1955, 76pp, 7s6d).

APRIL, 1956

CLINICAL: M Allgower offers neat study of *The Cellular Basis of Wound Repair* (CC Thomas, Springfield, Ill, 1956, 125pp, $5). A Bencini & PL Parola propose rhythmic insufflation of pericardium (pneumomassage) in cardiac arrest (*Surgery* 39/375/56). EB Hay & R Huggins discuss hemodynamics of Na dehydrocholate in shock by increasing arterial blood flow to liver (ibid p 199). E Kokas & Co find guanylic acid derivative from embryonic heart with ATP increases muscular efficiency & inhibits cardiac & adrenal work hypertrophy (*Acta Physiol Acad Scient Hungarica* 7/409/55). DM Little considers *Controlled Hypotension in Anesthesia & Surgery* (CC

Thomas, Springfield, Ill, 1956, 159pp, $6). MB Rosenblatt reviews *Cancer of the Lung: Pathology, Diagnosis & Treatment*, Oxford Press, New York, 1956, 330pp, $15). KE Schaefer & Co discuss physiology of skin diving & oxygen toxicity in underwater swimming (*J Appl Physiol* 8/519,524/56). S Standard & H Nathan ask *Should the Patient Know the Truth?* (Interscience, London, 1955, 160pp, 15s). LT Webster Jr & CS Davidson find carbonic anhydrase inhibitor "Diamox" may induce hepatic coma in susceptible alcoholics with cirrhosis (*Proc Soc Exp Biol Med* 91/27/56).

PSYCHOPHARMACOLOGY: EV Evarts finds bufotenine & LSD cause sensory disorder & quietness in monkeys without loss of muscle power (*Arch Neurol Psychiat* 75/49/56). R Hernandez-Peon & Co note effect of attention on electrical activity of cochlear nucleus (*Science* 123/331/Feb 24/56). BF Hoffman & Co propose quantitative evaluation of sleep (*J Appl Physiol* 8/361/56). E Jacobsen & Y Skaarup find diethylamino-ethylbenzilate HCl ("Suavitil"; benactyzine) an anticholinergic sedative (Acta Pharmacol Tox 11/124,147/55), & I Munkvad notes its value in allaying irritation in neuroses (Acta Psychi Neur Sc 30/729/55). NS Kline edits important symposium on *Psychopharmacology* (Am Asso Adv Sci, Washington DC, 1956, 165pp, $3.5). R Mangold & Co study cerebral circulation & metabolism in sleep & in prolonged wakefulness (J Clin Invest 34/1092/55). Our BH Marks notes barbiturates inhibit acetylation (*Science* 123/332/Feb 24/56). WG Mitchell & RC Grubbs find CO_2 inhibits audiogenic convulsions (*Science* 123/223/Feb 10/56). BE Schwarz & Co study behavioral & EEG effects of psychogenic drugs given intracisternally (*Arch Neurol Psychiat* 75/83/56). AW Wase & Co find turnover of brain phospholipids reduced by Chlorpromazine (*Arch Neurol Psychiat* 75/54/56). RHL Wilson & Co show diurnal rates of alcohol metabolism, with fall in sleep (*J Appl Physiol* 8/556/56). JZ Young ably reviews organization within nerve cells (*Endeavor* 15/5/56).

BIOLOGICAL: JAV Butler reviews facts & theories on effects of ionizing radiation on biological materials (*Rad Res* 4/20/56). PW Durbin & Co find lanthanons not absorbed orally, but after parenteral injection fixed in bone & liver, with slow release from bone (*Proc Soc Exp Biol Med* 91/78/56). KH Gustavson surveys *The Chemistry & Reactivity of Collagen* (Academic Press, New York, 1956, 342pp, $8). I Innerfield shows proteolytic enzymes reduce chemically induced inflammation by restoring homeostatic membrance factors (*Surgery* 39/426/56). AW Johnson & A Todd indicate complex porphyrine structure of vitamin B_{12} (*Endeavor* 15/29/56). EA Kabat considers *Blood Group Substances: Their Chemistry & Immunochemistry* (Academic Press, New York, 1956, 330pp, $8). AR Korey & JI Nurnberger edit *Neurochemistry* (PB Hoeber, NY, 1956, 250pp, $6.75). A Meister & PR Downey find vitamin B_6 participates in glutamine transaminase system & that isonicotine acid hydrazide depletes vitamin B_6 body reserves (*Proc Soc Exp Biol Med* 91/49/56). H Precht & Co look into *Temperatur und Leben* (Springer, Berlin, 1955, 526pp, DM 78). JJJG Prick & Co review *Thallium Poisoning* (Cleaver-Hume, London, 1955, 154pp, 19s). W Ruhland edits *Encyclopedia of Plant Physiology*, with Vol 3, *Water Relations of Plants*, edited by O Stocker now available (Springer, Berlin, 1956, 1140pp, DM 199). AM Shanes edits *Electrolytes*

in Biological Systems (Amer Physiol Soc, Washington DC, 1956, 244pp, $4). O Warburg pontificates on the origin of cancer cells (***Science*** 123/309/Feb 24/56). F Wassermann discusses intercellular components of connective tissue (***Ergeb Anat Entwgesch*** 35/254/56).

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